# PCT

# WORED INTELLECTUAL PROPERTY ORGANIZATION OF POTTING

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7:

(11) International Publication Number:

WO 00/42215

C12Q 1/68

A1

(43) International Publication Date:

20 July 2000 (20.07.00)

(21) International Application Number:

PCT/AU00/00008

(22) International Filing Date:

7 January 2000 (07.01.00)

(30) Priority Data:

PP 8078

8 January 1999 (08.01.99)

AU

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**Published** 

With international search report.

(54) Title: METHOD AND POLYNUCLEOTIDES FOR DETERMINING TRANSLATIONAL EFFICIENCY OF A CODON

(57) Abstract

A method is disclosed for determining the translational efficiency of an individual codon in a cell. The method comprises introducing into the cell a synthetic construct comprising a reporter polynucleotide fused in frame with a tandem repeat of said individual codon, wherein said reporter polynucleotide encodes a reporter protein, and wherein said synthetic construct is operably linked to a regulatory polynucleotide and measuring expression of said reporter protein in said cell to determine the translational efficiency of said codon.

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# METHOD AND POLYNUCLEOTIDES FOR DETERMINING TRANSLATIONAL EFFICIENCY OF A CODON

# FIELD OF THE INVENTION

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THIS INVENTION relates generally to gene expression and method and polynucleotides а particular, to in determining codon utilization in particular cells or tissues More particularly, the method organism. concerned with invention of the are polynucleotides ascertaining codon preferences in cells or tissues for the purpose of modifying the translational efficiency of proteinencoding polynucleotides in those cells or tissues.

### BACKGROUND OF THE INVENTION

It is well known that a "triplet" codon of four possible nucleotide bases can exist in 64 variant forms. These forms provide the message for only 20 different amino acids (as well as translation initiation and termination) and this means that some amino acids can be encoded by more than one codon. Some amino acids have as many as six "redundant", alternative codons while some others have a single, required codon.

For reasons not completely understood, codon utilization is highly biased in that alternative codons are not at all uniformly present in the endogenous DNA of differing cell types. In this regard, there appears to exist a variable natural hierarchy of "preference" for certain codons between different cell types or between different organisms.

Codon usage patterns have been shown to correlate with relative abundance of isoaccepting transfer RNA (iso-tRNA) species, and with genes encoding proteins of high versus low abundance. Moreover, the present inventors recently

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discovered that the intracellular abundance of different isotrnAs varies in different cells or tissues of a single multicellular organism (see copending International Application No. PCT/AU98/00530).

The implications of codon preference phenomena on gene expression are manifest in that these phenomena can affect the translational efficiency of messenger RNA (mRNA). It is widely known in this regard that translation of "rare codons", for which the corresponding iso-tRNA is in relatively low abundance, may cause a ribosome to pause during translation which can lead to a failure to complete a nascent polypeptide chain and an uncoupling of transcription and translation.

A primary goal in recombinant research is to provide transgenic organisms with expression of a foreign gene in an amount sufficient to confer the desired phenotype to the organism. However, expression of the foreign gene may be severely impeded if a particular host cell of the organism or the organism itself has a low abundance of iso-tRNAs corresponding to one or more codons of the foreign gene. Accordingly, a major aim of investigators in this field is to first ascertain the codon preference for particular cells or tissues in which a foreign gene is to be expressed, and to subsequently alter the codon composition of the foreign gene for optimized expression in those cells or tissues.

Codon preference may be determined simply by analyzing the frequency at which codons are used by genes expressed in a particular cell or tissue or in a plurality of cells or tissues of a given organism. Codon frequency tables as well as suitable methods for determining frequency of codon usage in an organism are described, for example, in an article by Sharp et al (1988, Nucleic Acids Res. 16 8207-8211). The relative level of gene expression (e.g., detectable protein expression) can provide

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an indirect measure of the relative abundance of specific iso-tRNAs expressed in different cells or tissues.

Alternatively, codon preference may be determined by measuring the relative intracellular abundance of different iso-tRNA species. For example, reference may be made to copending International Application No. PCT/AU98/00530 that describes a method that utilizes labeled oligonucleotides specific for different iso-tRNAs to probe an RNA extract prepared from a particular cell or tissue source.

The above methods provide useful indirect evidence for determining codon preference. However, such indirect evidence may not provide an accurate indication of the translational efficiency of a given codon. Accordingly, there is a need to provide a method that more directly ascertains the translational efficiency of a codon in a cell or tissue.

# SUMMARY OF THE INVENTION

In one aspect of the invention, there is provided a method for determining the translational efficiency of an individual codon in a cell, said method comprising:

- introducing into said cell a synthetic construct comprising a reporter polynucleotide fused in frame with a tandem repeat of said individual codon, wherein said reporter polynucleotide encodes a reporter protein, and wherein said synthetic construct is operably linked to a regulatory polynucleotide; and
- measuring expression of said reporter protein in said cell to determine the translational efficiency of said codon.
  - Preferably, the method further comprises comparing:

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- expression of said reporter protein in a cell to which a synthetic construct comprising a tandem repeat of said individual codon was provided; and
- expression of said reporter protein in a cell to which a synthetic construct comprising a tandem repeat of another individual codon was provided;

to thereby determine the relative translational efficiency of said individual codons in said cell.

Suitably, the method further comprises comparing:

- expression of said reporter protein in a cell to 10 which a synthetic construct comprising a tandem repeat of said individual codon was provided; and

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- expression of said reporter protein in another cell to which a synthetic construct comprising a tandem repeat of said individual codon was provided;

to thereby determine the translational efficiency of said individual codon in said cell relative to said other cell.

Preferably, the method further comprises:

- synthetic construct into - introducing the progenitor cell of said cell; and
  - producing said cell from said progenitor cell; wherein said cell contains said synthetic construct. Suitably, the method further comprises:
- synthetic construct into - introducing the progenitor of said cell; and
- organism or part thereof from - growing an progenitor cell;

wherein said organism comprises said cell containing said synthetic construct.

Suitably, the method further comprises: 30

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- introducing the synthetic construct into an organism or part thereof such that said synthetic construct is introduced into said cell.

In another aspect, the invention resides in a synthetic construct comprising a reporter polynucleotide fused in frame with a tandem repeat of individual codons, wherein said reporter polynucleotide encodes a reporter protein, and wherein said synthetic construct is operably linked to a regulatory polynucleotide.

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In yet another aspect of the invention, there is provided an improved method of constructing a synthetic polynucleotide from which a protein is selectively expressible in a target cell of an organism, relative to another cell of the organism, said method comprising:

- selecting a first codon of a parent polynucleotide for replacement with a synonymous codon which has a higher translational efficiency in said target cell than in said other cell; and
- replacing said first codon with said synonymous codon to form said synthetic polynucleotide, wherein said first codon and said synonymous codon are selected by:
  - comparing translational efficiencies of individual codons in said target cell relative to said other cell using the method broadly described above; and
  - selecting said first codon and said synonymous codon based on said comparison.

Preferably, said synonymous codon corresponds to a reporter construct from which the reporter protein is expressed in said target cell at a level that is at least 110%, preferably at least 200%, more preferably at least 500%, and most preferably at least 1000%, of that expressed from said reporter construct in said other cell.

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In a further aspect, the invention provides an improved method of constructing a synthetic polynucleotide from which a protein is expressible in a target cell of an organism at a higher level than from a parent polynucleotide encoding said protein, said method comprising:

- selecting a first codon of the parent polynucleotide for replacement with a synonymous codon which has a higher translational efficiency in said target cell than said first codon;
- 10 replacing said first codon with said synonymous codon to form said synthetic polynucleotide, wherein said first codon and said synonymous codon are selected by:
  - comparing translational efficiencies of different individual codons in said target cell using the method broadly described above; and
  - selecting said first codon and said synonymous codon based on said comparison.

Suitably, said synonymous codon corresponds to a reporter construct from which the reporter protein is expressed in said target cell at a level that is at least 110%, preferably at least 200%, more preferably at least 500%, and most preferably at least 1000%, of that expressed from the different reporter construct corresponding to said first codon.

### DETAILED DESCRIPTION

### 1. Definitions

The articles "a" and "an" are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

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Throughout this specification, unless the context requires otherwise, the words "comprise", "comprises" and "comprising" will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements.

By "expressible" is meant expression of a protein to a level sufficient to effect a particular function associated with the protein. By contrast, the terms "not expressible" and "not substantially expressible" as used interchangeably herein refers to (a) no expression of a protein, (b) expression of a protein to a level that is not sufficient to effect a particular function associated with the protein, (c) expression of a protein, which cannot be detected by a monoclonal antibody specific for the protein, or (d) expression of a protein, which is less that 1% of the level expressed in a wild-type cell that normally expresses the protein.

By "expressing said synthetic construct" is meant 20 transcribing the synthetic construct such that mRNA is produced.

By "expression vector" is meant any autonomous genetic element capable of directing the synthesis of a protein encoded by the vector. Such expression vectors are known by practitioners in the art.

As used herein, the term "function" refers to a biological, enzymatic, or therapeutic function.

By "highly expressed genes" is meant genes that express high levels of mRNA, and preferably high level of protein, relative to other genes.

By "isoaccepting transfer RNA" or "iso-tRNA" is meant one or more transfer RNA molecules that differ in their

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anticodon nucleotide sequence but are specific for the same amino acid.

By "natural gene" is meant a gene that naturally encodes the protein. However, it is possible that the parent polynucleotide encodes a protein that is not naturally-occurring but has been engineered using recombinant techniques.

The term "non-cycling cell" as used herein refers to a cell that has withdrawn from the cell cycle and has entered the GO state. In this state, it is known that transcription of endogenous genes and protein translation are at substantially reduced levels compared to phases of the cell cycle, namely G1, S, G2 and M. By contrast, the term "cycling cell" as used herein refers to a cell, which is in one of the above phases of the cell cycle.

By "obtained from" is meant that a sample such as, for example, a polynucleotide extract or polypeptide extract is isolated from, or derived from, a particular source of the host. For example, the extract can be obtained from a tissue or a biological fluid isolated directly from the host.

The term "oligonucleotide" as used herein refers to a polymer composed of a multiplicity of nucleotide residues ribonucleotides, related or (deoxyribonucleotides or structural variants or synthetic analogues thereof) linked via phosphodiester bonds (or related structural variants or Thus, while the synthetic analogues thereof). "oligonucleotide" typically refers to a nucleotide polymer in which the nucleotide residues and linkages between them are naturally occurring, it will be understood that the term also includes within its scope various analogues including, but nucleic acids restricted to, peptide phosphoramidates, phosphorothioates, methyl phosphonates, 2-O-methyl ribonucleic acids, and the like. The exact size of particular molecule can vary depending on the the

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application. An oligonucleotide is typically rather short in length, generally from about 10 to 30 nucleotide residues, but the term can refer to molecules of any length, although the term "polynucleotide" or "nucleic acid" is typically used for large oligonucleotides.

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By "operably linked" is meant that transcriptional and translational regulatory polynucleotides are positioned relative to a polypeptide-encoding polynucleotide in such a manner that the polynucleotide is transcribed and the polypeptide is translated.

By "pharmaceutically-acceptable carrier" is meant a solid or liquid filler, diluent or encapsulating substance that can be safely used in topical or systemic administration to a mammal.

"Polypeptide", "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues and to variants and synthetic analogues of the same. Thus, these terms apply to amino acid polymers in which one or more amino acid residues is a synthetic non-naturally occurring amino acid, such as a chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally-occurring amino acid polymers.

The term "polynucleotide" or "nucleic acid" as used herein designates mRNA, RNA, cRNA, cDNA or DNA. The term typically refers to oligonucleotides greater than 30 nucleotide residues in length.

By "primer" is meant an oligonucleotide which, when paired with a strand of DNA, is capable of initiating the synthesis of a primer extension product in the presence of a suitable polymerizing agent. The primer is preferably single-stranded for maximum efficiency in amplification but can alternatively be double-stranded. A primer must be sufficiently long to prime the synthesis of extension products in the presence of the polymerization agent. The

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length of the primer depends on many factors, including application, temperature to be employed, template reaction conditions, other reagents, and source of primers. example, depending on the complexity of the target sequence, the oligonucleotide primer typically contains 15 to 35 or more nucleotide residues, although it can contain fewer Primers can be large polynucleotides, nucleotide residues. 200 nucleotide residues to from about such as selected to can be or more. Primers kilobases "substantially complementary" to the sequence on the template to which it is designed to hybridize and serve as a site for "substantially Ву of synthesis. initiation t.he complementary", it is meant that the primer is sufficiently complementary to hybridize with a target polynucleotide. Preferably, the primer contains no mismatches with template to which it is designed to hybridize but this is not nucleotide example, non-complementary For residues can be attached to the 5' end of the primer, with the remainder of the primer sequence being complementary to the template. Alternatively, non-complementary nucleotide stretch of non-complementary nucleotide residues or a residues can be interspersed into a primer, provided that the primer sequence has sufficient complementarity with sequence of the template to hybridize therewith and thereby form a template for synthesis of the extension product of the primer.

"Probe" refers to a molecule that binds to a specific sequence or sub-sequence or other moiety of another molecule. Unless otherwise indicated, the term "probe" typically refers to a polynucleotide probe that binds to another polynucleotide, often called the "target polynucleotide", through complementary base pairing. Probes can bind target polynucleotides lacking complete sequence complementarity with the probe, depending on the stringency of the

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hybridization conditions. Probes can be labeled directly or indirectly.

The terms "precursor cell or tissue" and "progenitor cell or tissue" as used herein refer to a cell or tissue that can gives rise to a particular cell or tissue in which protein expression is to be targeted or in which translational efficiency of a codon is to be determined.

By "recombinant polypeptide" is meant a polypeptide made using recombinant techniques, i.e., through the expression of a recombinant or synthetic polynucleotide.

"Stringency" as used herein, refers to the temperature and ionic strength conditions, and presence or absence of certain organic solvents, during hybridization. The higher the stringency, the higher will be the degree of complementarity between immobilized polynucleotides and the labeled polynucleotide.

"Stringent conditions" refers to temperature and ionic conditions under which only polynucleotides having a high hybridize. The complementary bases will of stringency required is nucleotide sequence dependent components present various depends upon the hybridization. Generally, stringent conditions are selected to be about 10 to 20°C lower than the thermal melting point  $(T_m)$  for the specific sequence at a defined ionic strength The  $T_{m}$  is the temperature (under defined ionic strength and pH) at which 50% of a target sequence hybridizes to a complementary probe.

The term "synthetic polynucleotide" as used herein refers to a polynucleotide formed in vitro by the manipulation of a polynucleotide into a form not normally found in nature. For example, the synthetic polynucleotide can be in the form of an expression vector. Generally, such expression vectors include transcriptional and translational

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regulatory polynucleotide operably linked to the polynucleotide.

The term "synonymous codon" as used herein refers to a codon having a different nucleotide sequence than another codon but encoding the same amino acid as that other codon.

By "translational efficiency" is meant the efficiency of a cell's protein synthesis machinery to incorporate the amino acid encoded by a codon into a nascent polypeptide chain. This efficiency can be evidenced, for example, by the rate at which the cell is able to synthesize the polypeptide from an RNA template comprising the codon, or by the amount of the polypeptide synthesized from such a template.

polynucleotide molecule, a "vector" is meant preferably a DNA molecule derived, for example, from bacteriophage, or plant virus, into which Α inserted or cloned. polynucleotide can be preferably contains one or more unique restriction sites and can be capable of autonomous replication in a defined host cell including a target cell or tissue or a progenitor cell or tissue thereof, or be integrable with the genome of the defined host such that the cloned sequence is reproducible. Accordingly, the vector can be an autonomously replicating i.e., a vector that exists as an extrachromosomal which is independent of the replication entity, chromosomal replication, e.g., a linear or closed circular plasmid, an extrachromosomal element, a minichromosome, or an The vector can contain any means for artificial chromosome. assuring self-replication. Alternatively, the vector can be one which, when introduced into the host cell, is integrated replicated together with and genome chromosome(s) into which it has been integrated. system can comprise a single vector or plasmid, two or more vectors or plasmids, which together contain the total DNA to be introduced into the genome of the host cell,

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transposon. The choice of the vector will typically depend on the compatibility of the vector with the host cell into which the vector is to be introduced. The vector can also include a selection marker such as an antibiotic resistance selection of for be used that can transformants. Examples of such resistance genes are known to those of skill in the art and include the nptII gene that confers resistance to the antibiotics kanamycin and G418 (Geneticin®) and the hph gene which confers resistance to the antibiotic hygromycin B.

### 2. Method of the invention

The present invention is based, at least in part, the discovery that different but synonymous stretches identical codons fused respectively in frame with a reporter polynucleotide can give rise to different levels of reporter Not wishing to protein expressed within a given cell type. be bound by any particular theory, it is believed that a tandem series of identical codons causes a ribosome to pause during translation if the iso-tRNA corresponding to the identical codons is limiting. In this regard, it is known that ribosomal pausing leads to a failure to complete a nascent polypeptide chain and an uncoupling of transcription and translation. Accordingly, the levels of reporter protein expressed in the different cells or tissues are sensitive to iso-tRNA abundance of the intracellular corresponding to the identical codons and, therefore, provide a direct correlation of a cell's or tissue's preference for This means, for example, that if translating a given codon. the levels of the reporter protein obtained in a cell or tissue type to which a synthetic construct having a tandem series of identical first codons is provided are lower than the levels expressed in the same cell or tissue type to which a different synthetic construct having a tandem series of identical second codons is provided (i.e., wherein the first

codons are different from, but synonymous with, the second codons), then it can be deduced that the cell or tissue has a higher preference for the second codon relative to the first codon with respect to translation. Put another way, the second codon has a higher translational efficiency compared to the first codon in the cell or tissue type.

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With regard to differential protein expression between different cell or tissue types, it will be appreciated that if the levels of the reporter protein obtained in a target cell or tissue type to which a synthetic construct having a tandem series of identical codons is provided are lower than the levels expressed in the another cell or tissue type to which the same synthetic construct is provided, then it can be deduced that the target cell or tissue has a higher preference for the codon relative to the other cell or tissue with respect to translation. Put another way, the codon has a higher translational efficiency in the target cell or tissue relative to the other cell or tissue type.

As used herein, expression of a protein in a tissue refers alternatively to expression of the protein within a cell of the tissue or production of the protein within a cell and export of the protein from the cell to, for example, the extracellular matrix of a tissue.

Suitably, the tandem repeat comprises at least three identical codons. Preferably, the tandem repeat comprises four identical codons, more preferably five or seven identical codons and most preferably six identical codons.

The tandem repeat can be fused at a location adjacent to, or within, the reporter polynucleotide. The location is preferably selected such that the tandem repeat interferes with translation of at least a detectable portion of the reporter protein such that expression of the protein can be detected or assessed. Preferably, the tandem repeat is

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located immediately upstream (translationally) from the reporter polynucleotide.

It is of course possible that a tandem repeat of identical amino acid residues (e.g., an oligo-proline repeat) can render the reporter protein unstable. Typically, protein instability is detected when expression of the reporter gene is not detectable with any choice of isoaccepting codon specific for the amino acid corresponding to the tandem repeat. The inventors have found in this regard that protein instability can be alleviated by use of at least one spacer codon within the tandem repeat of identical codons, wherein the spacer codon encodes a neutral amino acid.

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Preferably, a spacer codon is efficiently translated in the cell or tissue type relative to other synonymous codons. This is important so that translation of the spacer codon is not rate limiting. The neutral amino acid includes, but is not restricted to, alanine and glycine.

The reporter polynucleotide can encode any suitable protein for which expression can be detected directly or indirectly such as by suitable assay. Suitable reporter polynucleotides include, but are not restricted to, polynucleotides encoding  $\beta$ -galactosidase, firefly luciferase, alkaline phosphatase, chloramphenicol acetyltransferase

(CAT),  $\beta\text{-glucuronidase}$  (GUS), herbicide resistance genes such as the bialophos resistance (BAR) gene that confers resistance to the herbicide BASTA, and green fluorescent protein (GFP). Assays for the activities associated with such proteins are known by those of skill in the art. Preferably, the reporter polynucleotide encodes GFP.

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Persons of skill in the art will appreciate that reporter polynucleotides need not correspond to a full-length gene encoding a particular reporter protein. In this regard, the invention also contemplates reporter polynucleotide subsequences encoding desired portions of a parent reporter protein, wherein an activity or function of the parent protein is retained in said portions. A polynucleotide subsequence encodes a domain of the reporter protein having an activity associated therewith and preferably encodes at least 10, 20, 50, 100, 150, or 500 contiguous amino acid residues of the reporter protein.

The instant method is applicable to any suitable cell or tissue type and, hence, is not restricted to application to mammalian cells/tissues. Accordingly, the cell or tissue type can be of any animal or plant origin. The cell or tissue type can be of any suitable lineage. For example, a suitable cell can include a eukaryotic cell, and preferably a cell or cell line capable of being grown in vitro. Suitable cell lines can include, for example, CV-1 cells, COS cells, yeast or spodoptera cells. The invention also contemplates cells that can be prokaryotic in origin.

Suitable methods for isolating particular cells or tissues are known to those of skill in the art. For example, one can take advantage of one or more particular characteristics of a cell or tissue to specifically isolate the cell or tissue from a heterogeneous population. Such characteristics include, but are not limited to, anatomical location of a tissue, cell density, cell size, cell

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morphology, cellular metabolic activity, cell uptake of ions such as Ca<sup>2+</sup>, K<sup>+</sup>, and H<sup>+</sup> ions, cell uptake of compounds such as stains, markers expressed on the cell surface, protein fluorescence, and membrane potential. Suitable methods that can be used in this regard include surgical removal of tissue, flow cytometry techniques such as fluorescence-activated cell sorting (FACS), immunoaffinity separation (e.g., magnetic bead separation such as Dynabead<sup>TM</sup> separation), density separation (e.g., metrizamide, Percoll<sup>TM</sup>, or Ficoll<sup>TM</sup> gradient centrifugation), and cell-type specific density separation.

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In an alternate embodiment, progenitor cells or tissues can be used for initially introducing the synthetic construct. Any suitable progenitor cell or tissue can be used which gives rise to a particular cell or tissue of interest for which codon preference is to be ascertained. For example, a suitable progenitor cell can comprise an undifferentiated cell. In the case of a plant, a suitable progenitor cell and tissue can include a meristematic cell and a callus tissue, respectively.

In another embodiment, the synthetic construct can be introduced first into an organism or part thereof before subsequent expression of the construct in a particular cell or tissue type. Any suitable organism is contemplated by the invention including unicellular and as multi-cellular organisms. Exemplary multi-cellular organisms include plants and animals such as mammals(e.g., humans).

The invention further provides a synthetic construct comprising a reporter polynucleotide fused in frame with a tandem repeat of (e.g., 2, 3, 4, 5, 6, or 7 or more) identical codons, wherein said reporter polynucleotide encodes a reporter protein, and wherein said synthetic construct is operably linked to one or more regulatory polynucleotides.

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The construction of the synthetic construct can be effected by any suitable technique. For example, in vitro mutagenesis methods can be employed, which are known to those Suitable mutagenesis methods are of skill in the art. described for example in the relevant sections of Ausubel, et (supra) and of Sambrook, et al., (supra) which are Alternatively, suitable incorporated herein by reference. methods for altering DNA are set forth, for example, in U.S. Patent Nos. 4,184,917, 4,321,365 and 4,351,901, which are Instead of in vitro incorporated herein by reference. mutagenesis, the synthetic construct can be synthesized de novo using readily available machinery. Sequential synthesis in U.S. Patent for example, described, DNA is reference. incorporated herein by 4,293,652, which is However, it should be noted that the present invention is not and not directed to, any one particular dependent on, technique for constructing the synthetic construct.

Regulatory polynucleotides which can be utilized to regulate expression of the synthetic construct include, but enhancer, an promoter, limited to, a are transcriptional terminator. Such regulatory polynucleotides The construct are known to those of skill in the art. Suitable promoter. preferably comprises at least one promoters that can be utilized to induce expression of the constitutive include invention polynucleotides the of promoters and inducible promoters.

The step of introducing the synthetic construct into a particular cell or tissue type, or into a progenitor cell or tissue thereof, or into an organism or part thereof for subsequent introduction into a particular cell or tissue will differ depending on the intended use and or species, and may involve lipofection, electroporation, micro-projectile bombardment infection by Agrobacterium tumefaciens or A rhizogenes, or protoplast fusion. Such methods are known to those skilled in the art.

Alternatively, the step of introduction may involve non-viral and viral vectors, cationic liposomes, retroviruses and adenoviruses such as, for example, described in Mulligan, R.C., (1993 *Science* **260** 926-932) which is incorporated herein by reference. Such methods may include:

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- A. Local application of the synthetic nucleic acid sequence by injection (Wolff et al., 1990, Science 247 1465-1468, is incorporated herein by reference), any other instillation or means. implantation, local combination with be used in may also implantation, injection, surgical application by instillation or any other means, of cells responsive to the reporter protein encoded by the synthetic construct. This method may also be used in combination with local implantation, surgical injection, application by of another factor or instillation or any other means, for the activity of said reporter required factors protein.
- B. General systemic delivery by injection of DNA, (Calabretta et al., 1993, Cancer Treat. Rev. 19 169-179, which is 20 incorporated herein by reference), or RNA, alone or combination with liposomes (Zhu et al., 1993, Science 261 209-212, which is incorporated herein by reference), viral capsids or nanoparticles (Bertling et al., 1991, Biotech. Appl. Biochem. 13 390-405, which is incorporated herein by 25 reference) or any other mediator of delivery. targeting might be achieved by linking the synthetic construct to a targeting molecule (the so-called "magic bullet" approach employing for example, an antibody), or by local application by injection, surgical implantation 30 or any other means, of another factor or factors required for the activity of the protein produced from said synthetic construct, or of cells responsive said to reporter protein.

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C. Injection or implantation or delivery by any means, of cells that have been modified ex vivo by transfection (for example, in the presence of calcium phosphate: Chen et al., 1987, Mole. Cell Biochem. 7 2745-2752, or of cationic lipids and polyamines: Rose et al., 1991, BioTech. 10 520-525, which articles are incorporated herein by reference), infection, injection, electroporation (Shigekawa et al., 1988, BioTech. 6 742-751, which is incorporated herein by so as to increase any other. wav reference) or expression of said synthetic construct in those cells. mediated plasmid, by modification may be The adenoviral bacteriophage, cosmid, viral (such as retroviral; Mulligan, 1993, Science 260 926-932; Miller, 1992, Nature 357 455-460; Salmons et al., 1993, Hum. Gen. Ther. 4 129-141, which articles are incorporated herein by other agents or other or vectors, reference) modification such as liposomes (Zhu et al., 1993, Science 261 209-212, which is incorporated herein by reference), viral capsids or nanoparticles (Bertling et al., 1991, Biotech. Appl. Biochem. 13 390-405, which is incorporated mediator reference), anv other or by modification. The use of cells as a delivery vehicle for genes or gene products has been described by Barr et al., 1991, Science 254 1507-1512 and by Dhawan et al., 1991, Science 254 1509-1512, which articles are incorporated herein by reference. Treated cells may be delivered in combination with any nutrient, growth factor, matrix or other agent that will promote their survival in the treated subject.

Advantageously, the translational efficiencies of different codons may be determined by comparing expression of the reporter protein in a given cell or tissue type or between different cell or tissue types. One of ordinary skill in the art will thereby be able to determine a "codon"

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preference table" for one or more cells or tissues. Comparison of codon preference tables relating to different cell or tissue types may be used to identify codons for tailoring a synthetic polynucleotide to target expression of a protein to a particular cell or tissue, as described hereinafter. Comparison of codons within a codon preference table for a particular cell or tissue type can be used to identify codons for tailoring a synthetic polynucleotide to express a protein at higher or lower levels in that cell or tissue type than a parent polynucleotide, as described hereinafter.

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The invention further contemplates cells or tissues containing therein the synthetic construct of the invention, or alternatively, cells or tissues produced from the method of the invention.

3. Synthetic polynucleotides for targeting protein expression to a particular cell or tissue

invention also provides an improved method of constructing a synthetic polynucleotide from which a protein is selectively expressible in a target cell of an organism, relative to another cell of the organism. This method is in disclosed the method on based part International application PCT/AU98/00530 (the entire contents of which are hereby incorporated by reference) in which a first codon of a parent polynucleotide is replaced with a synonymous codon which has a higher translational efficiency in said target cell than in said other cell. method of the invention is characterized by selecting the by comparing translational and synonymous codons target said efficiencies of individual codons in using the method broadly relative to said other cell described is Section 2.

# 3.1. Selection of synonymous and first codons

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The present method preferably includes the step of selecting the codons such that the synonymous codon has a higher translational efficiency in said target cell or tissue ("cell or tissue" is sometimes referred to herein as "cell/tissue") relative to said one or more other cells or tissues.

A method for determining translational efficiencies of different codons in and between different cells or tissues is The translational described in detail in Section 2. efficiencies so determined can be used to identify which isocoding triplets are differentially translated between the In a typical scenario, there different cells or tissues. will be: (A) codons with higher translational efficiencies in to one or more relative target cell/tissue higher translational codons with cells/tissues; (B) efficiencies in the one or more other cells/tissues relative to the target cell/tissue; and (C) codons with about the same translational efficiencies in the target cell/tissue relative to the one or more other cells/tissues. Synonymous codons (A) codons. are selected such that they correspond to Preferably, a synonymous codon is selected such that it has the largest difference in translational efficiency in the tissue relative to the existing codon or (sometimes referred to as a "first codon") that it replaces. Existing codons in a parent polynucleotide are preferably selected such that they do not have the same translational bias as the synonymous codons with respect to the target cell/tissue and the one or more other cell/tissue (i.e., existing codons should preferably not correspond to (A) existing codons have can codons). However, translational efficiencies in each of the target cell/tissue and the one or more other cells/tissues (i.e., existing They can also have a codons can correspond to (C) codons. translational bias opposite to that of the synonymous codons

(i.e., existing codons can, and preferably do, correspond to (B) codons).

Suitably, a synonymous codon has a translational efficiency in the target cell/tissue that is at least 110%, preferably at least 200%, more preferably at least 500%, and still more preferably at least 1000%, of that in the other cell(s)/tissue(s). In the case of two or more synonymous codons having similar translational efficiencies in the target cell/tissue relative to the other cell(s)/tissue(s), it will be appreciated that any one of these codons can be used to replace the existing codon.

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It is preferable but not necessary to replace all the existing codons of the parent polynucleotide with synonymous codons having higher translational efficiencies in the target cell/tissue compared to the other cells/tissues. Increased expression can be accomplished even with partial replacement. Suitably, the replacement step affects 5%, 10%, 15%, 20%, 25%, 30%, more preferably 35%, 40%, 50%, 60%, 70% or more of the existing codons of the parent polynucleotide.

The difference in level of protein expressed in the target cell/tissue from a synthetic polynucleotide relative to that expressed in the other cell(s)/tissue(s) depends on the percentage of existing codons replaced by synonymous codons, and the difference in translational efficiencies of the synonymous codons in the target cell/tissue relative to the other cell(s)/tissue(s). Put another way, the fewer such difference in smaller the replacements, and/or the translational efficiencies of the synonymous between the different cells/tissues, the smaller the difference in protein expression between the target cell/tissue and the other cell(s)/tissue(s) will be. Conversely, the more such the difference and/or the greater replacements, translational efficiencies of the synonymous codons between the different cells/tissues, the greater the difference in

protein expression between the target cell/tissue and the other cell(s)/tissue(s) will be. The inventors have found in this respect that a protein can be expressed from a synthetic polynucleotide in a target cell/tissue at levels greater than 10,000-fold over those expressed in another cell/tissue.

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In a preferred embodiment, the synonymous codon is a codon which has a higher translational efficiency in the target cell or tissue relative to a precursor cell or tissue of the target cell or tissue.

In an alternate embodiment, the synonymous codon is a codon which has a higher translational efficiency in the target cell or tissue relative to a cell or tissue derived from said target cell or tissue.

The two codons can be selected by measuring translational efficiencies of different codons in the target cell or tissue relative to the one or more other cells or tissues and identifying the at least one existing codon and the synonymous codon based on this measurement.

Suitably, the synonymous codon corresponds to a reporter construct from which the reporter protein is expressed in said target cell at a level that is at least 110%, preferably at least 200%, more preferably at least 500%, and most preferably at least 1000%, of that expressed from the said reporter construct in said other cell.

# 3.2. Construction of synthetic polynucleotides

The step of replacing a synonymous codon for said first codon in a parent polynucleotide may be effected by any suitable technique. For example, in vitro mutagenesis methods may be employed as for example discussed in Section 2.

It is not necessary to replace all the first codons of the parent polynucleotide with synonymous codons each corresponding to a codon that has a higher translational

efficiency in the target cell relative to said other cell. Increased expression may be accomplished even with partial replacement. Preferably, the replacing step affects 5%, 10%, 15%, 20%, 25%, 30%, more preferably 35%, 40%, 50%, 60%, 70% or more of the existing codons of the parent nucleic acid sequence.

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The parent polynucleotide is preferably a natural gene.

The parent polynucleotide may be obtained from a plant Alternatively, the parent polynucleotide may or an animal. any other eukaryotic organism from obtained In a preferred embodiment, the parent prokaryotic organism. polynucleotide is obtained from a pathogenic organism. such a case, a natural host of the pathogenic organism is preferably a plant or animal. For example, the pathogenic organism may be a yeast, bacterium or virus. However, it will be understood that the parent polynucleotide need not be obtained from the organism in which a protein is to be expressed but may be obtained from any suitable source such as from another eukaryotic or prokaryotic organism.

Suitable proteins which may be used for selective expression in accordance with the invention include, but are not limited to the cystic fibrosis transmembrane conductance regulator (CFTR) protein, and adenosine deaminase (ADA). In the case of CFTR, a parent nucleic acid sequence encoding the CFTR protein which may be utilized to produce the synthetic nucleic acid sequence is described, for example, in Riordan et al (1989, Science 245 1066-1073), and in the GenBank database under Accession No. HUMCFTRM, which are incorporated herein by reference.

Regulatory polynucleotides which may be utilized to regulate expression of the synthetic polynucleotide include, but are not limited to, a promoter, an enhancer, and a transcriptional terminator. Such regulatory polynucleotides are known to those of skill in the art. The construct

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preferably comprises at least one promoter. Suitable promoters that can be utilized to induce expression of the synthetic polynucleotides of the invention include constitutive promoters and inducible promoters.

Synthetic polynucleotides according to the invention may be operably linked to one or more regulatory sequences in the form of an expression vector.

The invention also contemplates synthetic polynucleotides encoding one or more desired portions of the protein to be expressed. A polynucleotide encodes a domain of the protein having a function associated therewith, or which is otherwise detectable, and preferably encodes at least 10, 20, 50, 100, 150, or 500 contiguous amino acid residues of the protein.

# 15 4. Synthetic polynucleotides for enhanced protein expression in a particular cell or tissue

In contrast to differential protein expression between be appreciated that it will different cells/tissues, synthetic polynucleotide may be tailored with synonymous codons such that expression of a protein in a target cell is In this regard, the difference in level of protein from а synthetic target cell/tissue the expressed in polynucleotide relative to that expressed from a parent polynucleotide depends on the percentage of existing codons and the difference codons, synonymous by translational efficiencies between the existing codons and the synonymous codons in the target cell/tissue. Put another way, the fewer such replacements, and/or the smaller the efficiencies between translational difference in synonymous and existing codons, the smaller the difference in protein expression between the synthetic polynucleotide and Conversely, the more such parent polynucleotide will be. difference in the greater replacements, and/or the

translational efficiencies between the synonymous and existing codons, the greater the difference in protein expression between the synthetic polynucleotide and parent polynucleotide will be. The inventors have found in this respect that a protein can be expressed from a synthetic polynucleotide in a target cell/tissue at levels greater than 10,000-fold than from a parent polynucleotide.

Preferably, the at least one existing codon and the synonymous codon are selected such that said protein is expressed from said synthetic polynucleotide in said target cell or tissue at a level which is at least 110%, preferably at least 200%, more preferably at least 500%, and most preferably at least 1000%, of that expressed from said parent polynucleotide in said target cell or tissue.

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The invention is further described with reference to the following non-limiting examples.

# EXAMPLE 1

Construction of expression vectors for determining relative codon preferences in mammalian cells.

Synthetic gfp genes were constructed in which a single artificial start codon (ATG) followed by a stretch of five identical codons is fused in frame immediately upstream of a gfp coding sequence. A reverse oligonucleotide primer (SEQ ID NO:185; sequence complementary to the termination codon of suite underlined), and а is oligonucleotide primers (SEQ ID NO: 126 through 184; the first codon of GFP, is underlined) were synthesized and used for PCR amplification of a humanized gfp gene (SEQ ID NO:124) (GIBCO) as template with Taq DNA polymerase (Amplification parameters: 95°C/30 sec; 52°C/30 sec; 72°C/1 min; 30 cycles). The amplified fragments have nucleic acid sequences and deduced amino acid sequences as shown in SEQ ID NO:1 through 124.

In summary, the synthetic fragments contain an artificial start codon followed by a tandem repeat of five identical codons specific for a given iso-tRNA species. The tandem repeat immediately precedes the second codon of the gfp gene. The synthetic fragments by SEQ ID NO, and encoded tandem repeat, are presented in the TABLE 1.

The amplified fragments were cloned between the *Eco*RI and *Kpn*I sites of the mammalian expression vector pCDNA3 containing SV40 ori (Invitrogen) and the CMV promoter.

### Transfection of COS-1 cells

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COS-1 cells were grown continuously in DMEM media supplemented with 10% fetal calf serum (FCS), glutamine, penicillin and streptomycin. Cells were passaged from a 150 Cells cm<sup>2</sup> flasks. into multiple 25 flask transfected using a QIAGEN Effectene™ transfection kit (and instructions, incorporated herein manufacturer's reference) when confluency of the cells was between 60-80%. Briefly, 1  $\mu g$  of plasmid DNA was diluted into 10  $\mu L$  of filtered TE buffer and 140  $\mu L$  of QIAGENTM Buffer EC. Eight microliters of QIAGEN™ Enhancer was added followed by vortexing and incubation at room temperature for 2-5 min. QIAGEN<sup>TM</sup> Effectene (10  $\mu L$ ) was added followed by vortexing for 10 seconds and a further incubation at room temperature The cells were washed once in 1x PBS followed by for 10 min. After 48 hrs, cells re-suspension in fresh media (1 mL). were harvested and washed in 1x PBA (phosphate buffered saline plus azide). Cells adhering to the flask were removed Cells were then filtered by scraping with a cell scraper. through a 70  $\mu m$  filter before addition of 300  $\mu L$  of 2% paraformaldehyde and 300  $\mu L$  of 10x FCS. Cells were kept on ice in the dark until FACS analysis.

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Synthetic gfp mRNA expression of transfected cells was tested by reverse transcriptase PCR. GFP protein expression was analyzed by confocal microscopy and flow cytometry.

### Confocal microscopy

Transfected COS-1 cells were examined using a Bio-Rad MRC-600 laser-scanning confocal microscope equipped with a krypton-argon laser and filter sets suitable for the detection of fluorescein and Texas red dyes (Bio-Rad K1yK2), and a Nikon 603 PlanApo<sup>TM</sup> numerical aperture 1.2 water-immersion objective. Dual-channel confocal images and video montages of the transfected cells can be suitably composed using ADOBE PhotoShop<sup>TM</sup>.

### Flow cytometry

Transfected COS-1 cells were analyzed with a Becton Dickinson™ Flow cytometer Elite II. Omega Filters™ allowed detection of green fluorescence emission (EMI510/20 - collects light from 490-530 nm) and yellow fluorescence emission (EM2 550/30 - collects light form 525-580 nm) from the transfected cells.

### 20 Results

A series of 64 reporter constructs (see TABLE 1) was made and validated, in which the *gfp* gene is preceded in frame by a tandem repeat of 5 identical codons. Together, the series covers the entire set of isoaccepting codon triplets.

The series was transfected into a single cell line, and expression levels measured by flow cytometry (see TABLE 2). Overall, the expression level of the reporter gene constructs in the cell line varied over a range of 20-fold, according to the triplet used in the reporter construct. Repeated determinations on the same construct showed excellent interassay reproducibility ( $r^2 = 0.9$ ). Variation in expression

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levels across the isoaccepting codons for a single amino acid ranged from 1.4-fold for valine to 13-fold for threonine, with a median of about 4-fold. Variation in expression between amino acids was of the same order of magnitude. The order of magnitude of the effect is defined as an average of 4 fold per amino acid if 5 copies are incorporated, compatible with an extreme in range of expression levels of up to  $(1.6)^{200} = 10^{86}$  over an average 200-amino acid residues protein. This figure is derived as:

[1 + ((4-1)( range of reporter construct expression)/
5 (no of triplets in the reporter construct))]<sup>200 (no of amino acid)</sup>
residues in the protein)

and is more than sufficient to explain the observed differences in expression of mammalian genes according to codon usage.

The results presented in TABLE 2 also show that various codons in the undifferentiated epithelial cells (COS-1) have translational efficiencies at least two-fold higher or twotheir corresponding relative to those of lower Representative codons having at least a synonymous codons. two-fold higher translational efficiency relative to at least one of their corresponding synonymous codons include aga (Arg), cgg (Arg), tgc (Cys), gga (Gly), ggc (Gly), ccg (Pro), cga (Pro), aca (Thr), acg (Thr), and act (Thr). Thus, these codons appear to be preferred for translation contrast, cells. Ву epithelial undifferentiated least a two-fold lower representative codons having at translational efficiency relative to at least one of their corresponding synonymous codons include agg (Arg), tgt (Cys), ggg (Gly), ggt (Gly), ccc (Pro), cct (Pro), and acc (Thr). These latter codons would therefore appear to be preferred for translation in the undifferentiated epithelial Accordingly, if higher protein expression is required cells. within undifferentiated epithelial cells such as COS-1 cells,

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the preferred codons should be used to replace any existing codons of a parent polynucleotide encoding the protein that In this respect, a correspond to the less preferred codons. protein for increasing substitution algorithm codon epithelial cells non-differentiated expression in presented in TABLE 3. However, if lower protein expression is required in non-differentiated epithelial cells, the less preferred codons should be used to replace any existing codons of the parent polynucleotide that correspond to the preferred codons.

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The disclosure of every patent, patent application, and publication cited herein is hereby incorporated by reference in its entirety.

The present invention has been described in terms of particular embodiments found or proposed by the present inventors to comprise preferred modes for the practice of the Those of skill in the art will appreciate that, invention. in light of the present disclosure, numerous modifications and changes can be made in the particular embodiments scope of departing the from exemplified without intended to All such modifications are invention. included within the scope of the appended claims.

### TABLE 1

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Synthetic gfp constructs are tabulated by SEQ ID NO and by the codon corresponding to the tandem repeat of five identical codons immediately upstream of the gfp gene.

#### TABLE 2

Mean fluorescence intensities of up to four different samples of transiently transfected COS-1 cells are shown (Green mean 1-4). Synthetic gfp constructs are tabulated by SEQ ID NO and by the codon corresponding to the tandem repeat immediately upstream of the gfp gene.

#### TABLE 3

Input codons and output codons represent, respectively, synonymous codons and existing (i.e., "first") codons according to the invention. Change means an actual change of a codon.

# TABLES

TABLE 1 Synthetic fragments and tandem repeats encoded thereby.

			<del></del>		
SEQ ID NO	Tandem repeat	SEQ ID NO	Tandem repeat		
1	Ala (GCA) x 5	65	Leu (CTT) x 5		
3	Ala (GCC) x 5	. 67	Leu (TTA) x 5		
5	Ala (GCG) x 5	69	Leu (TTG) x 5		
7	Ala (GCT) x 5	71	Lys (AAA) x 5		
9	Arg (AGA) x 5	73	Lys (AAG) x 5		
11	Arg (AGG) x 5	75	Phe (TTT) x 5		
13	Arg (CGA) x 5	77	Phe (TTC) x 5		
15	Arg (CGC) x 5	79	Pro (CCC) x 5		
17	Arg (CGG) x 5	81	Pro (CCG) x 5		
19	Arg (CGT) x 5	83	Pro (CCT) x 5		
21	Asn (AAC) x 5	85	Pro (CGA) x 5		
23	Asn (AAT) x 5	87	Ser (AGC) x 5		
25	Asp (GAC) x 5	89	Ser (AGT) x 5		
27	Asp (GAT) x 5	91	Ser (TCA) x 5		
29	Cys (TGC) x 5	93	Ser (TCC) x 5		
31	Cys (TGT) x 5	95	Ser (TCG) x 5		
33	Gln (CAA) x 5	97	Ser (TCT) x 5		
35	Gln (CAG) x 5	99	Thr (ACA) x 5		
37	Gly (GAA) x 5	101	Thr (ACC) x 5		
39	Gly (GAG) x 5	103	Thr (ACG) x 5		
41	Gly (GGA) x 5	105	Thr (ACT) x 5		

SEQ ID NO	Tandem repeat	SEQ ID NO	Tandem repeat
43	Gly (GGC) x 5	107	Trp (TGG) x 5
45	Gly (GGG) × 5	109	Tyr (TAT) x 5
47	Gly (GGT) x 5	111	Tyr (TAC) x 5
49	His (CAC) x 5	113	Val (GTA) x 5
51	His (CAT) x 5	115	Val (GTC) x 5
53	Ile (ATA) x 5	. 117	Val (GTG) x 5
55	Ile (ATC) x 5	119	Val (GTT) x 5
57	Ile (ATT) x 5	121	Stop (TAA) x 5
59	Leu (CTA) x 5	122	Stop (TAG) x 5
61	Leu (CTC) x 5	123	Stop (TGA) x 5
63	Leu (CTG) x 5	124	control

SEQ ID	Codon	[DNA]	Green	Green	Green	Green	Average
NO		(µg/mL)	mean	mean	mean	mean	
			1	2	3	4	
1	Ala (GCA)	1.07	45.70	54.40			50.05
3	Ala (GCC)	1.10	43.70	50.00			46.85
5	Ala (GCG)	0.03	28.50	42.40			35.45
7	Ala (GCT)	0.56	11.60	48.30			29.95
9	Arg (AGA)	0.90	29.00	33.00			31.00
11	Arg (AGG)	0.34	7.35	2.88			5.12
13	Arg (CGA)	1.00	18.30	14.20			16.25
15	Arg (CGC)	0.86	14.60	16.00			15.30
17	Arg (CGG)	1.00	22.50	20.60			21.55
19	Arg (CGT)	0.68	21.70	32.20			26.95
21	Asn (AAC)	0.02	<u> </u>				
23	Asn (AAT)	0.38	28.30	8.22			18.26
25	Asp (GAC)	0.46	24.90	17.80			21.35
27	Asp (GAT)	1.39	14.50	18.90			16.70
29	Cys (TGC)	0.68	21.90	16.10			19.00
31	Cys (TGT)	1.14	5.95	5.89			5.92
33	Gln (CAA)	0.28	26.50	43.50			35.00
35	Gln (CAG)	1.98	44.70	48.60			46.65
37	Glu (GAA)	0.60	10.30	22.70			16.50
39	Glu (GAG)	0.43	3.86				
41	Gly (GGA)	0.33	28.80	36.30	1		32.55
43	Gly (GGC)	1.62	17.80	28.10			22.95
45	Gly (GGG)	1.15	6.43	4.96			5.70
47	Gly (GGT)	1.39	7.12	4.02			5.57
49	His (CAC)	1.62	29.90	39.70			34.80
51	His (CAT)	1.69	43.40	37.20			40.30
53	Ile (ATA)	0.69	2.76	3.98			3.37

SEQ ID	Codon	[DNA]	Green	Green	Green	Green	Average
NO		(µg/mL)	mean	mean	mean	mean	
			1	2	3	4	
55	Ile (ATC)	1.52	4.12	2.83			3.48
57	Ile (ATT)	1.77	3.19	3.16			3.18
59	Leu (CTA)	0.10	15.00	3.01	5.26	2.44	6.43
61	Leu (CTC)	1.74	2.70	2.92	2.56		2.73
63	Leu (CTG)	0.41	2.80	7.51	2.63		4.31
65	Leu (CTT)	1.43	3.17	3.56	2.70		3.14
67	Leù (TTA)	0.62	3.85	3.91	2.66		3.47
69	Leu (TTG)	0.70	2.87	4.63	2.85		' 3.45
71	Lys (AAA)	0.10	11.90	8.24			10.07
73	Lys (AAG)	0.56	19.20	16.00			17.60
75	Phe (TTT)	2.28	2.67				
77	Phe (TTC)	1.65	4.35				
79	Pro (CCC)	0.40	12.00	8.95			10.48
81	Pro (CCG)	0.13	17.40	25.40			21.40
83	Pro (CCT)	0.40	10.60	9.89		-	10.25
85	Pro (CGA)	0.17	27.20	34.80			31.00
87	Ser (AGC)	0.03	62.40				
89	Ser (AGT)	0.81	23.10				
91	Ser (TCA)	0.08	30.70	37.20			33.95
93	Ser (TCC)	1.68	32.90				
95	Ser (TCG)	1.58	60.00		1		
97	Ser (TCT)	0.62	26.80	40.70			33.75
99	Thr (ACA)	1.70	37.80	39.90			38.85
101	Thr (ACC)	7.69	3.48	2.75			3.12
103	Thr (ACG)	1.06	36.10	44.10			40.10
105	Thr (ACT)	1.42	38.80	42.60			40.70
107	Trp (TGG)	1.19	5.21	4.29			4.75
109	Tyr (TAT)	0.02					
111	Tyr (TAC)	1.07	12.00	15.00			13.50
113	Val (GTA)	0.16	10.50	3.81			7.16
115	Val (GTC)	0.66	15.20	4.55	3.65	5.06	7.12
117	Val (GTG)	0.10	9.17	4.29	7.03	2:36	5.71

SEQ ID	Codon	[DNA]	Green	Green	Green	Green	Average
NO		(µg/mL)	mean	mean	mean	mean	
			1	2	3	4	*
119	Val (GTT)	0.49	14.10	2.63	3.70	2.49	5.73
121	stop	1.88	39.40	35.30			37.35
	(TAA)						
122	stop	2.86	2.88	3.28			3.08
	(TAG)						
123	stop	0.02					
	(TGA)						
124			9.34	61.60	30.40	55.00	39.09
GFP							
alone							
control			2.33	2.21	2.16	2.00	2.18

TABLE 3

differentiated epithelial cells

Substitution algorithm used for high level expression in non-

Input Codon	Output Codon	Amino Acid	Change
AAA	AAG	LYS	Yes
AAC	AAC	ASN	No
AAG	AAG	LYS	No
ААТ	AAC	ASN	Yes
AAU .	AAC	ASN	Yes
ACA	ACC	THR	Yes
ACC	ACC	THR	No
ACG	ACC	THR	Yes
ACT	ACC	THR	Yes
ACU	ACC	THR	Yes
AGA	AGG	ARG	Yes
AGC	AGC	SER	No
AGG	AGG	ARG	No
AGT	AGC	SER	Yes
AGU	AGC	SER	Yes
ATA	ATC	ILE	Yes
ATC	ATC	ILE	No
ATG	ATG	MET	No
ATT	ATC	ILE	Yes
AUA	ATC	ILE	Yes
AUC	ATC	ILE	No
AUG	ATG	MET	No
DUA	ATC	ILE	Yes
CAA	CAG	GLN	Yes
CAC	CAC	HIS	No
CAG	CAG	GLN	No
CAT	CAC	HIS	Yes
CAU	CAC	HIS	Yes
CCA	ccc	PRO	Yes
ccc	ccc	PRO	No
CCG	ccc	PRO	Yes
ССТ	ccc	PRO	Yes
ccu	ccc	PRO	Yes
CGA	CGC	ARG	Yes

Input Codon	Output Codon	Amino Acid	Change
CGC	CGC	ARG	No
CGG	CGC	ARG	Yes
CGT	CGC	ARG	Yes
CGÜ	CGC	ARG	Yes
CTA	CTG	LEU	Yes
CTC	CTG	LEU	Yes
CTG	CTG	LEU	No
СТТ	CTG	LEU	Yes
CUA	CTG	LEU	Yes
CUC	CTG	TEO.	Yes
CUG	CTG	LEU	No
CUU	ĊТG	LEU	Yes
GAA	GAG	GLU	Yes
GAC	GAC	ASP	No
GAG	GAG	. GLU	No
GAT	GAC	ASP	Yes
GAU	GAC	ASP	Yes
GCA	GCC	ALA	Yes
GCC	GCC	ALA	No
GCG	GCC	ALA	Yes
GCT	GCC	ALA	Yes
GCU	GCC	ALA	Yes
GGA	GGC	GLY	Yes
GGC	GGC	GLY	No
GGG	GGG	GLY	No
GGT	GGC	GLY	Yes
GGU	GGC	GLY	Yes
GTA	GTG	VAL	Yes
GTC	GTG	VAL	Yes
GTG	GTG	VAL	No
GTT	GTG	VAL	Yes
GUA	GTG	VAL	Yes
GUC	GTG	VAL	Yes
GUG	GTG	VAL	No
GUU	GTG	VAL	Yes
TAA	TAA	XXX	No
TAC	TAC	TYR	No
TAG	TAG	XXX	No
TAT	TAC	TYR	Yes
TCA	TCC	SER	Yes

Input Codon	Output Codon	Amino Acid	Change
TCC	TCC	SER	No
TCG	TCC	SER	Yes <sup>.</sup>
TCT	TCC	SER	Yes
TGA	TGA	XXX	No
TGC	TGC	CYS	No
TGG	TGG	TRP	No
TGT	TGT	CYS	No
TTA	CTG	LEU	Yes
TTC	TTC	PHE	No
TTG	CTG	LEU	Yes
TTT	TTC	PHE	No
UAA	TAA	xxx	No
UAC	TAC	TYR	No
UAG	TAG	xxx	No
UAU	TAC	TYR	Yes
UCA	TCC	SER	Yes
บСС	TCC	SER	No ·
UCG	TCC	SER	Yes
ບເບ	TCC	SER	Yes
UGA	TGA	xxx	No
UGC	TGC	CYS	. No
UGG	TGG	TRP	No
บGบ	TGT	CYS	No
AUU	CTG	LEU	Yes
υυς	TTC	PHE	No
บบG	CTG	LEU	Yes
טטט	TTC	PHE	Yes

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## WHAT IS CLAIMED IS:

- 1. A method for determining the translational efficiency of an individual codon in a cell, said method comprising:
  - introducing into said cell a synthetic construct comprising a reporter polynucleotide fused in frame with a tandem repeat of said individual codon, wherein said reporter polynucleotide encodes a reporter protein, and wherein said synthetic construct is operably linked to a regulatory polynucleotide; and
- 10 measuring expression of said reporter protein in said cell to determine the translational efficiency of said codon.
  - 2. The method of claim 1, further comprising comparing:
- expression of said reporter protein in a cell to
   which a synthetic construct comprising a tandem repeat of said individual codon was provided; and
  - expression of said reporter protein in a cell to which a synthetic construct comprising a tandem repeat of another individual codon was provided;
- to thereby determine the relative translational efficiency of said individual codons in said cell.
  - 3. The method of claim 1, further comprising comparing:
    - expression of said reporter protein in a cell to which a synthetic construct comprising a tandem repeat of said individual codon was provided; and
    - expression of said reporter protein in another cell to which a synthetic construct comprising a tandem repeat of said individual codon was provided;
  - to thereby determine the translational efficiency of said individual codon in said cell relative to said other cell.

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- 4. The method of claim 1, further comprising:
  - introducing the synthetic construct into a progenitor cell of said cell; and
    - producing said cell from said progenitor cell;
- 5 wherein said cell contains said synthetic construct.
  - 5. The method of claim 1, further comprising
    - introducing the synthetic construct into a progenitor
      of said cell; and
- growing an organism or part thereof from said
  10 progenitor cell;

wherein said organism comprises said cell containing said synthetic construct.

- 6. The method of claim 1, further comprising
- introducing the synthetic construct into an organism
   or part thereof such that said synthetic construct is introduced into said cell.
  - 7. A synthetic construct comprising a reporter polynucleotide fused in frame with a tandem repeat of individual codons, wherein said reporter polynucleotide encodes a reporter protein, and wherein said synthetic construct is operably linked to a regulatory polynucleotide.
  - 8. A vector comprising the synthetic construct of claim 7.
  - 9. A cell comprising the synthetic construct of claim 7.
  - 10. A cell comprising the vector of claim 9.
- 25 11. An improved method of constructing a synthetic polynucleotide from which a protein is selectively expressible in a target cell of an organism, relative to another cell of the organism, said method comprising:
- selecting a first codon of a parent polynucleotide
   for replacement with a synonymous codon which has a higher

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translational efficiency in said target cell than in said other cell; and

- replacing said first codon with said synonymous codon to form said synthetic polynucleotide, wherein said first codon and said synonymous codon are selected by:

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- comparing translational efficiencies of individual codons in said target cell relative to said other cell; and
- selecting said first codon and said synonymous codon based on said comparison, wherein said comparison comprises;
- introducing into said target cell and said other cell a synthetic construct comprising a reporter polynucleotide fused in frame with a tandem repeat of an individual codon, wherein said reporter polynucleotide encodes a reporter protein, and wherein said synthetic construct is operably linked to a regulatory polynucleotide; and
- comparing expression of said reporter protein in
   said target cell relative to said other cell;

to thereby determine the translation efficiency of individual codons in said target cell relative to said other cell.

- 12. The method of claim 11, wherein said synonymous codon corresponds to a reporter construct from which the reporter protein is expressed in said target cell at a level that is at least 110% of that expressed from the said reporter construct in said other cell.
- 13. An improved method of constructing a synthetic polynucleotide from which a protein is expressible in a target cell of an organism at a higher level than from a parent polynucleotide expressing said protein, said method comprising:

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- selecting a first codon of the parent polynucleotide for replacement with a synonymous codon which has a higher translational efficiency in said target cell than said first codon;
- replacing said first codon with said synonymous codon to form said synthetic polynucleotide, wherein said first codon and said synonymous codon are selected by:

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- comparing translational efficiencies of different individual codons in said target cell; and
- selecting said first codon and said synonymous codon based on said comparison wherein said comparison comprises:
- introducing into a target cell a synthetic construct comprising a reporter polynucleotide fused in frame with a tandem repeat of an individual codon, wherein said reporter polynucleotide encodes a reporter protein, and wherein said synthetic construct is operably linked to a regulatory polynucleotide;
- introducing into a target cell a different synthetic construct comprising the reporter polynucleotide fused in frame with a tandem repeat of another individual codon; and
- comparing expression of said reporter protein from each synthetic construct in said target cell;
- to thereby determine the translation efficiency of individual codons in said target cell.
  - 14. The method of claim 13, wherein said synonymous codon corresponds to a reporter construct from which the reporter protein is expressed in said target cell at a level that is at least 110% of that expressed from the different reporter construct corresponding to said first codon.

- 15. A synthetic polynucleotide constructed according to the method of claim 11 or claim 13.
- 16. A vector comprising the synthetic polynucleotide of claim
  15.
- 5 17. A cell comprising the synthetic polynucleotide of claim 15.
  - 18. A cell comprising the vector of claim 16.

## SEQUENCE LISTING

<110> The University of Queensland (all designated States except US) Frazer, Ian Hector and Zhou, Jian (US only)
<120> METHOD AND POLYNUCLEOTIDES FOR DETERMINING TRANSLATIONAL EFFICIENCY OF A CODON
<130> Codon optimization
<140> Not yet assigned <141> Herewith
<150> PP8078 <151> 1999-01-08
<160> 180
<170> PatentIn Ver. 2.0
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tct gtc agc gga gag ggt gaa ggt gat gcc aca tac gga aag ctc acc 144 Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr 35 40 45
ctg aaa ttc atc tgc acc act gga aag ctc cct gtg cca tgg cca aca 192 Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr 50 55 60
ctg gtc act acc ttc tct tat ggc gtg cag tgc ttt tcc aga tac cca Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 65 70 75 80
gac cat atg aag cag cat gac ttt ttc aag agc gcc atg ccc gag ggc 288 Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly 85 90 95
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acc cgc gct gaa gtc aag ttc gaa ggt gac acc ctg gtg aat aga atc 384

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Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His 140 Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 150 Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr 200 Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val 215 Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 235 230 Leu Tyr Lys <210> 3 <211> 732 <212> DNA <213> Artificial Sequence <223> Description of Artificial Sequence: Ala(GCC)5GFP <220> <221> CDS <222> (1) .. (732) atg gcc gcc gcc gcc agc aag ggc gag gaa ctg ttc act ggc gtg 48 Met Ala Ala Ala Ala Ser Lys Gly Glu Glu Leu Phe Thr Gly Val 10 gtc cca att ctc gtg gaa ctg gat ggc gat gtg aat ggg cac aaa ttt 96 Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe 144 tct gtc agc gga gag ggt gaa ggt gat gcc aca tac gga aag ctc acc Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr ctg aaa ttc atc tgc acc act gga aag ctc cct gtg cca tgg cca aca 192 Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr 55 240 ctg gtc act acc ttc tct tat ggc gtg cag tgc ttt tcc aga tac cca Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 70 gac cat atg aag cag cat gac ttt ttc aag agc gcc atg ccc gag ggc 288 Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly 336 tat gtg cag gag aga acc atc ttt ttc aaa gat gac ggg aac tac aag Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys

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atc Ile	ggc Gly	gac Asp 195	ggc Gly	cct Pro	gtg Val	ctc Leu	ctc Leu 200	cca Pro	gac Asp	aac Asn	cat His	tac Tyr 205	ctg Leu	tcc Ser	acc Thr	624
cag Gln	tct Ser 210	gcc Ala	ctg Leu	tct Ser	aaa Lys	gat Asp 215	ccc Pro	aac Asn	gaa Glu	aag Lys	aga Arg 220	gac Asp	cac His	atg Met	gtc Val	672
ctg Leu 225	Leu	gag Glu	ttt Phe	gtg Val	acc Thr 230	gct Ala	gct Ala	Gly	atc Ile	aca Thr 235	cat His	ggc Gly	atg Met	gac Asp	gag Glu 240	720
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	50	)				55					Val 60					
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Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 105 Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile 115 Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 150 Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 170 Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro 185 Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val 215 Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 230 Leu Tyr Lys <210> 9 <211> 732 <212> DNA <213> Artificial Sequence <223> Description of Artificial Sequence: Arg(AGA)5GFP <220> <221> CDS <222> (1)..(732) <400> 9 atg aga aga aga aga agc aag ggc gag gaa ctg ttc act ggc gtg 48 Met Arg Arg Arg Arg Ser Lys Gly Glu Glu Leu Phe Thr Gly Val gtc cca att ctc gtg gaa ctg gat ggc gat gtg aat ggg cac aaa ttt 96 Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe 20 144 tct gtc agc gga gag ggt gaa ggt gat gcc aca tac gga aag ctc acc Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr ctg aaa ttc atc tgc acc act gga aag ctc cct gtg cca tgg cca aca 192 Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr ctg gtc act acc ttc tct tat ggc gtg cag tgc ttt tcc aga tac cca

Leu	Val	Thr	Thr	Phe		Tyr	Gly	Val	Gln	Cys	Phe	Ser	Arg	Tyr	Pro 80	
65			~		70	<b>73.</b>	+++	++0	aag	75	acc	atq	CCC	gag		288
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Ser	Val	Ser 35		Glu	Gly	Glu	Gly 40		Ala	Thr	Tyr	Gly 45	Lys	Leu	Thr	
Leu	Lys 50		lle	. Cys	Thr	Thr 55		Lys	Leu	Pro	Val 60	Pro	Trp	Pro	Thr	

Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His 135 Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 170 Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys <210> 11 <211> 732 <212> DNA <213> Artificial Sequence <223> Description of Artificial Sequence: Arg(AGG)5GFP <220> <221> CDS <222> (1)..(732) <400> 11 48 atg agg agg agg agg agc aag ggc gag gaa ctg ttc act ggc gtg Met Arg Arg Arg Arg Ser Lys Gly Glu Glu Leu Phe Thr Gly Val 96 gtc cca att ctc gtg gaa ctg gat ggc gat gtg aat ggg cac aaa ttt Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe 25 tct gtc agc gga gag ggt gaa ggt gat gcc aca tac gga aag ctc acc 144 Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr ctg aaa ttc atc tgc acc act gga aag ctc cct gtg cca tgg cca aca 192 Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr

60 50 55 ctg gtc act acc ttc tct tat ggc gtg cag tgc ttt tcc aga tac cca Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 65 gac cat atg aag cag cat gac ttt ttc aag agc gcc atg ccc gag ggc 288 Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly 90 tat gtg cag gag aga acc atc ttt ttc aaa gat gac ggg aac tac aag 336 Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 105 384 acc cgc gct gaa gtc aag ttc gaa ggt gac acc ctg gtg aat aga atc Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile 120 432 gag ctg aag ggc att gac ttt aag gag gat gga aac att ctc ggc cac Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His 135 480 aag ctg gaa tac aac tat aac tcc cac aat gtg tac atc atg gcc gac Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 155 150 aag caa aag aat ggc atc aag gtc aac ttc aag atc aga cac aac att 528 Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile gag gat gga tcc gtg cag ctg gcc gac cat tat caa cag aac act cca 576 Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro 180 185 624 atc ggc gac ggc cct gtg ctc ctc cca gac aac cat tac ctg tcc acc Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr 200 195 cag tot goo otg tot aaa gat ooc aac gaa aag aga gac cac atg gto 672 Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val 215 210 720 ctg ctg gag ttt gtg acc gct gct ggg atc aca cat ggc atg gac gag Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 225 732 ctg tac aag tga Leu Tyr Lys <210> 12 <211> 243 <212> PRT <213> Artificial Sequence <400> 12 Met Arg Arg Arg Arg Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe

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Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr 35 40 45

Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro 185 Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val 220 Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 225 Leu Tyr Lys <210> 13 <211> 732 <212> DNA <213> Artificial Sequence <223> Description of Artificial Sequence: Arg(CGA)5GFP <221> CDS <222> (1)..(732) <400> 13 48 atg cga cga cga cga agc aag ggc gag gaa ctg ttc act ggc gtg Met Arg Arg Arg Arg Ser Lys Gly Glu Glu Leu Phe Thr Gly Val gtc cca att ctc gtg gaa ctg gat ggc gat gtg aat ggg cac aaa ttt 96 Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe 20 tct gtc agc gga gag ggt gaa ggt gat gcc aca tac gga aag ctc acc Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr

ctg a Leu I	aaa Lys 50	ttc Phe	atc Ile	tgc Cys	acc Thr	act Thr 55	gga Gly	aag Lys	ctc Leu	cct Pro	gtg Val 60	cca Pro	tgg Trp	cca Pro	aca Thr	192
ctg o Leu V 65	gtc Val	act Thr	acc Thr	ttc Phe	tct Ser 70	tat Tyr	ggc Gly	gtg Val	cag Gln	tgc Cys 75	ttt Phe	tcc Ser	aga Arg	tac Tyr	cca Pro 80	240
gac d Asp H	cat His	atg Met	aag Lys	cag Gln 85	cat His	gac Asp	ttt Phe	ttc Phe	aag Lys 90	agc Ser	gcc Ala	atg Met	ccc Pro	gag Glu 95	ggc Gly	288
tat o Tyr N	gtg Val	cag Gln	gag Glu 100	aga Arg	acc Thr	atc Ile	ttt Phe	ttc Phe 105	aaa Lys	gat Asp	gac Asp	ggg Gly	aac Asn 110	tac Tyr	aag Lys	336
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gag ( Glu <i>i</i>	gat Asp	gga Gly	tcc Ser 180	gtg Val	cag Gln	ctg Leu	gcc Ala	gac Asp 185	cat His	tat Tyr	caa Gln	cag Gln	aac Asn 190	act Thr	cca Pro	576
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ctg Leu : 225	ctg Leu	gag Glu	ttt Phe	gtg Val	acc Thr 230	gct Ala	gct Ala	ggg Gly	atc Ile	aca Thr 235	cat His	ggc Gly	atg Met	gac Asp	gag Glu 240	720
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Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile 120 . Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His 135 Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 145 Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 170 Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr 205 200 Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 235 230 Leu Tyr Lys <210> 15 <211> 732 <212> DNA <213> Artificial Sequence <223> Description of Artificial Sequence: Arg(CGC)5GFP <220> <221> CDS <222> (1)..(732) <400> 15 atg cgc cgc cgc cgc agc aag ggc gag gaa ctg ttc act ggc gtg 48 Met Arg Arg Arg Arg Ser Lys Gly Glu Glu Leu Phe Thr Gly Val 96 gtc cca att ctc gtg gaa ctg gat ggc gat gtg aat ggg cac aaa ttt Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe

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ctg Leu	aaa Lys 50	ttc Phe	atc Ile	tgc Cys	acc Thr	act Thr 55	gga Gly	aag Lys	ctc Leu	cct Pro	gtg Val 60	cca Pro	tgg Trp	cca Pro	aca Thr	192
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gac Asp	cat His	atg Met	aag Lys	cag Gln 85	cat His	gac Asp	ttt Phe	ttc Phe	aag Lys 90	agc Ser	gcc Ala	atg Met	ccc Pro	gag Glu 95	ggc Gly	288
tat Tyr	gtg Val	cag Gln	gag Glu 100	aga Arg	acc Thr	atc Ile	ttt Phe	ttc Phe 105	aaa Lys	gat Asp	gac Asp	Gly	aac Asn 110	tac Tyr	aag Lys	336
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PCT/AU00/00008

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ctg Leu	aaa Lys 50	ttc Phe	atc Ile	tgc Cys	acc Thr	act Thr 55	gga Gly	aag Lys	ctc Leu	cct Pro	gtg Val 60	cca Pro	tgg Trp	cca Pro	aca Thr	192
ctg Leu 65	gtc Val	act Thr	acc Thr	ttc Phe	tct Ser 70	tat Tyr	ggc Gly	gtg Val	cag Gln	tgc Cys 75	ttt Phe	tcc Ser	aga Arg	tac Tyr	cca Pro 80	240
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acc Thr	cgc Arg	gct Ala 115	gaa Glu	gtc Val	aag Lys	ttc Phe	gaa Glu 120	ggt Gly	gac Asp	acc Thr	ctg Leu	gtg Val 125	aat Asn	aga Arg	atc Ile	384
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cag Gln	tct Ser 210	gcc Ala	ctg Leu	tct Ser	aaa Lys	gat Asp 215	Pro	aac Asn	gaa Glu	aag Lys	aga Arg 220	Asp	cac His	atg Met	gtc Val	672
ctg Leu 225	ctg Leu	gag Glu	ttt Phe	gtg Val	acc Thr 230	Ala	gct Ala	Gly	atc Ile	aca Thr 235	His	ggc Gly	atg Met	gac Asp	gag Glu 240	720
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<212> DNA

<213> Artificial Sequence

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gtc Val	cca Pro	att Ile	ctc Leu 20	gtg Val	gaa Glu	ctg Leu	gat Asp	ggc Gly 25	gat Asp	gtg Val	aat Asn	ggg Gly	cac His 30	aaa Lys	ttt Phe	96
tct Ser	gtc Val	agc Ser 35	gga Gly	gag Glu	ggt Gly	gaa Glu	ggt Gly 40	gat Asp	gcc Ala	aca Thr	tac Tyr	gga Gly 45	aag Lys	ctc Leu	acc Thr	144
ctg Leu	aaa Lys 50	ttc Phe	atc Ile	tgc Cys	acc Thr	act Thr 55	gga Gly	aag Lys	ctc Leu	cct Pro	gtg Val 60	cca Pro	tgg Trp	cca Pro	aca Thr	192
ctg Leu 65	gtc Val	act Thr	acc Thr	ttc Phe	tct Ser 70	tat Tyr	ggc Gly	gtg Val	cag Gln	tgc Cys 75	ttt Phe	tcc Ser	aga Arg	tac Tyr	cca Pro 80	240
gac Asp	cat His	atg Met	aag Lys	cag Gln 85	cat His	gac Asp	ttt Phe	ttc Phe	aag Lys 90	agc Ser	gcc Ala	atg Met	ccc Pro	gag Glu 95	ggc Gly	288
tat Tyr	gtg Val	cag Gln	gag Glu 100	aga Arg	acc Thr	atc Ile	ttt Phe	ttc Phe 105	aaa Lys	gat Asp	gac Asp	ggg Gly	aac Asn 110	tac Tyr	aag Lys	336
acc Thr	cgc Arg	gct Ala 115	gaa Glu	gtc Val	aag Lys	ttc Phe	gaa Glu 120	ggt Gly	gac Asp	acc Thr	ctg Leu	gtg Val 125	aat Asn	aga Arg	atc Ile	384
gag Glu	ctg Leu 130	aag Lys	ggc Gly	att Ile	gac Asp	ttt Phe 135	aag Lys	gag Glu	gat Asp	gga Gly	aac Asn 140	att Ile	ctc Leu	ggc Gly	cac His	432
aag Lys 145	ctg Leu	gaa Glu	tac Tyr	aac Asn	tat Tyr 150	aac Asn	tcc Ser	cac His	aat Asn	gtg Val 155	tac Tyr	atc Ile	atg Met	gcc Ala	gac Asp 160	480
aag Lys	caa Gln	aag Lys	aat Asn	ggc Gly 165	atc Ile	aag Lys	gtc Val	aac Asn	ttc Phe 170	aag Lys	atc Ile	aga Arg	cac His	aac Asn 175	att Ile	528
gag Glu	gat Asp	Glv	Ser	Val	Gln	Leu	Ala	gac Asp 185	His	Tyr	Gln	Gln	aac Asn 190	act Thr	cca Pro	576
atc Ile	ggc Gly	gac Asp 195	ggc Gly	cct Pro	gtg Val	ctc Leu	ctc Leu 200	cca Pro	gac Asp	aac Asn	cat His	tac Tyr 205	ctg Leu	tcc Ser	acc Thr	624
cag Gln	tct Ser 210	gcc Ala	ctg Leu	tct Ser	aaa Lys	gat Asp 215	ccc Pro	aac Asn	gaa Glu	aag Lys	aga Arg 220	gac Asp	cac His	atg Met	gtc Val	672
ctg Leu 225	ctg Leu	gag Gŀu	ttt Phe	gtg Val	acc Thr 230	gct Ala	gct Ala	ggg Gly	atc Ile	aca Thr 235	cat His	ggc Gly	atg Met	gac Asp	gag Glu 240	720
_	tac Tyr	_	tga													732

<210> 20 <211> 243 <212> PRT <213> Artificial Sequence

<400> 20 Met Arg Arg Arg Arg Ser Lys Gly Glu Glu Leu Phe Thr Gly Val  $1 \ 5$  .  $10 \ 15$ 

Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe 20 25 30

Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr 35 40 45

Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr 50 55 60

Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 65 70 . 75 80

Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly 85 90 95

Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 100 105 110

Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile 115 120 125

Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His

Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 145 150 155 160

Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 165 170 175

Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro 180 185 190

Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr 195 200 205

Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val 210 220

Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 225 230 235 240

Leu Tyr Lys

<210> 21

<211> 732

<212> DNA

<213> Artificial Sequence

:220>

<223> Description of Artificial Sequence: Asn(AAC)5GFP

<220>

<221> CDS

<222> (1)..(732)

	2+0	)> 21 aac Asn	220	aac Asn	aac Asn 5	aac Asn	agc Ser	aag Lys	ggc Gly	gag Glu 10	gaa Glu	ctg Leu	ttc Phe	act Thr	ggc Gly 15	gtg Val	48
	gtc Val	cca Pro	att Ile	ctc Leu 20	gtg Val	gaa Glu	ctg Leu	gat Asp	ggc Gly 25	gat Asp	gtg Val	aat Asn	Gly ggg	cac His 30	aaa Lys	ttt Phe	96
	tct Ser	gtc Val	agc Ser 35	gga Gly	gag Glu	ggt Gly	gaa Glu	ggt Gly 40	gat Asp	gcc Ala	aca Thr	tac Tyr	gga Gly 45	aag Lys	ctc Leu	acc Thr	144
	ctg Leu	aaa Lys 50	ttc Phe	atc Ile	tgc Cys	acc Thr	act Thr 55	gga Gly	aag Lys	ctc Leu	cct Pro	gtg Val 60	cca Pro	tgg Trp	cca Pro	aca Thr	192
	ctg Leu 65	gtc Val	act Thr	acc Thr	ttc Phe	tct Ser 70	tat Tyr	ggc Gly	gtg Val	cag Gln	tgc Cys 75	ttt Phe	tcc Ser	aga Arg	tac Tyr	cca Pro 80	240
	gac Asp	cat His	atg Met	aag Lys	cag Gln 85	cat His	gac Asp	ttt Phe	ttc Phe	aag Lys 90	agc Ser	gcc Ala	atg Met	ccc Pro	gag Glu 95	ggc Gly	288
	tat Tyr	gtg Val	cag Gln	gag Glu 100	aga Arg	acc Thr	atc Ile	ttt Phe	ttc Phe 105	aaa Lys	gat Asp	gac Asp	Gly	aac Asn 110	tac Tyr	aag Lys	336
	acc Thr	cgc Arg	gct Ala 115	gaa Glu	gtc Val	aag Lys	ttc Phe	gaa Glu 120	ggt Gly	gac Asp	acc Thr	ctg Leu	gtg Val 125	aat Asn	aga Arg	atc Ile	384
	gag Glu	ctg Leu 130	aag Lys	ggc Gly	att Ile	gac Asp	ttt Phe 135	aag Lys	gag Glu	gat Asp	gga Gly	aac Asn 140	att Ile	ctc Leu	ggc Gly	cac His	432
•	aag Lys 145	ctg Leu	gaa Glu	tac Tyr	aac Asn	tat Tyr 150	aac Asn	tcc Ser	cac His	aat Asn	gtg Val 155	tac Tyr	atc Ile	atg Met	gcc Ala	gac Asp 160	480
	Lys	Gln	Lys	Asn	Gly 165	Ile	Lys	Val	Asn	ttc Phe 170	Lys	Ile	Arg	HIS	175	ше	528
	Glu	Asp	Gly	Ser 180	Val	Gln	Leu	Ala	185	cat His	Tyr	GIn	GIN	190	1111	PIO	576
	atc Ile	ggc Gly	gac Asp 195	ggc Gly	cct Pro	gtg Val	ctc Leu	ctc Leu 200	Pro	gac Asp	aac Asn	cat His	tac Tyr 205	ctg Leu	tcc Ser	acc Thr	624
	cag Gln	tct Ser 210	Ala	ctg Leu	tct Ser	aaa Lys	gat Asp 215	Pro	aac Asn	gaa Glu	aag Lys	aga Arg 220	gac Asp	cac His	atg Met	gtc Val	672
	ctg Leu 225	Leu	gag Glu	ttt Phe	gtg Val	acc Thr 230	Ala	gct Ala	ggg	atc Ile	aca Thr 235	Hıs	ggc Gly	atg Met	gac Asp	gag Glu 240	720
		tac Tyr															732

<210> 22

<211> 243 <212> PRT

<213> Artificial Sequence

<400> 22

Met Asn Asn Asn Asn Ser Lys Gly Glu Glu Leu Phe Thr Gly Val

Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe

Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr

Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr

Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro

Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly

Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 105

Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile 120

Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His

Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp

Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 170

Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro

Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr 200

Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val 215

Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu

Leu Tyr Lys

<210> 23

<211> 732

<212> DNA

<213> Artificial Sequence

<223> Description of Artificial Sequence: Asn(AAT)5GFP

<220>

<221> CDS <222> (1)..(732)

<400	> 23	<b>.</b>														
atg Met 1	aat	aat	aat Asn	aat Asn 5	aat Asn	agc Ser	aag Lys	ggc Gly	gag Glu 10	gaa Glu	ctg Leu	ttc Phe	act Thr	ggc Gly 15	gtg Val	48
gtc Val	cca Pro	att Ile	ctc Leu 20	gtg Val	gaa Glu	ctg Leu	gat Asp	ggc Gly 25	gat Asp	gtg Val	aat Asn	ggg Gly	cac His 30	aaa Lys	ttt Phe	96
tct Ser	gtc Val	agc Ser 35	gga Gly	gag Glu	ggt Gly	gaa Glu	ggt Gly 40	gat Asp	gcc Ala	aca Thr	tac Tyr	gga Glỳ 45	aag Lys	ctc Leu	acc Thr	144
ctg Leu	aaa Lys 50	ttc Phe	atc Ile	tgc Cys	acc Thr	act Thr 55	gga Gly	aag Lys	ctc Leu	cct Pro	gtg Val 60	cca Pro	tgg Trp	cca Pro	aca Thr	192
ctg Leu 65	gtc Val	act Thr	acc Thr	ttc Phe	tct Ser 70	tat Tyr	ggc Gly	gtg Val	cag Gln	tgc Cys 75	ttt Phe	tcc Ser	aga Arg	tac Tyr	cca Pro 80	240
gac Asp	cat His	atg Met	aag Lys	cag Gln 85	cat His	gac Asp	ttt Phe	ttc Phe	aag Lys 90	agc Ser	gcc Ala	atg Met	ccc Pro	gag Glu 95	ggc Gly	288
tat Tyr	gtg Val	cag Gln	gag Glu 100	aga Arg	acc Thr	atc Ile	ttt Phe	ttc Phe 105	aaa Lys	gat Asp	gac Asp	Gly ggg	aac Asn 110	tac Tyr	aag Lys	336
acc Thr	cgc Arg	gct Ala 115	gaa Glu	gtc Val	aag Lys	ttc Phe	gaa Glu 120	ggt Gly	gac Asp	acc Thr	ctg Leu	gtg Val 125	aat Asn	aga Arg	atc Ile	384
gag Glu	ctg Leu 130	aag Lys	ggc Gly	att Ile	gac Asp	ttt Phe 135	aag Lys	gag Glu	gat Asp	gga Gly	aac Asn 140	att Ile	ctc Leu	ggc Gly	cac His	432
aag Lys 145	ctg Leu	gaa Glu	tac Tyr	aac Asn	tat Tyr 150	aac Asn	tcc Ser	cac His	aat Asn	gtg Val 155	tac Tyr	atc Ile	atg Met	gcc Ala	gac Asp 160	480
aag Lys	caa Gln	aag Lys	aat Asn	ggc Gly 165	atc Ile	aag Lys	gtc Val	aac Asn	ttc Phe 170	aag Lys	atc Ile	aga Arg	cac His	aac Asn 175	att Ile	528
gag Glu	gat Asp	gga Gly	tcc Ser 180	gtg Val	cag Gln	ctg Leu	gcc Ala	gac Asp 185	His	tat Tyr	caa Gln	cag Gln	aac Asn 190	act Thr	cca Pro	576
atc Ile	ggc Gly	gac Asp 195	ggc Gly	cct Pro	gtg Val	ctc Leu	ctc Leu 200	cca Pro	gac Asp	aac Asn	cat His	tac Tyr 205	ctg Leu	tcc Ser	acc Thr	624
cag Gln	tct Ser 210	gcc Ala	ctg Leu	tct Ser	aaa Lys	gat Asp 215	ccc Pro	aac Asn	gaa Glu	aag Lys	aga Arg 220	gac Asp	cac His	atg Met	gtc Val	672
ctg Leu 225	ctg Leu	gag Glu	ttt Phe	gtg Val	acc Thr 230	Ala	gct Ala	Gly	atc Ile	aca Thr 235	His	ggc Gly	atg Met	gac Asp	gag Glu 240	720

732

ctg tac aag tga Leu Tyr Lys

<210> 24

<211> 243

<212> PRT

<213> Artificial Sequence

Met Asn Asn Asn Asn Ser Lys Gly Glu Glu Leu Phe Thr Gly Val

Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe

Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr

Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr

Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 65 70 75 80

Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly

Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys

Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile

Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His

Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 150

Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 170

Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro

Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr 200

Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val 215

Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu

Leu Tyr Lys

<210> 25

<211> 732

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Asp(GAC)5GFP <220> <221> CDS <222> (1)..(732) <400> 25 48 atg gac gac gac gac agc aag ggc gag gaa ctg ttc act ggc gtg Met Asp Asp Asp Asp Ser Lys Gly Glu Glu Leu Phe Thr Gly Val gtc cca att ctc gtg gaa ctg gat ggc gat gtg aat ggg cac aaa ttt 96 Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe 20 tct gtc agc gga gag ggt gaa ggt gat gcc aca tac gga aag ctc acc 144 Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr 40 . 192 ctg aaa ttc atc tgc acc act gga aag ctc cct gtg cca tgg cca aca Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr 50 240 ctg gtc act acc ttc tct tat ggc gtg cag tgc ttt tcc aga tac cca Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 70 gac cat atg aag cag cat gac ttt ttc aag agc gcc atg ccc gag ggc 288 Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly tat gtg cag gag aga acc atc ttt ttc aaa gat gac ggg aac tac aag 336 Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 100 acc cgc gct gaa gtc aag ttc gaa ggt gac acc ctg gtg aat aga atc Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile 384 120 125 gag ctg aag ggc att gac ttt aag gag gat gga aac att ctc ggc cac 432 Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His aag ctg gaa tac aac tat aac tcc cac aat gtg tac atc atg gcc gac Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 480 150 145 aag caa aag aat ggc atc aag gtc aac ttc aag atc aga cac aac att 528 Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 165 gag gat gga tcc gtg cag ctg gcc gac cat tat caa cag aac act cca 576 Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro 190 180 185 624 atc ggc gac ggc cct gtg ctc ctc cca gac aac cat tac ctg tcc acc Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr 195 672 cag tot goo ctg tot aaa gat coo aac gaa aag aga gac cac atg gto Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val 215 210 720 ctq ctq qag ttt gtg acc gct gct ggg atc aca cat ggc atg gac gag

- xxvii -

Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 225 230 235 240

ctg tac aag tga Leu Tyr Lys 732

<210> 26

<211> 243

<212> PRT

<213> Artificial Sequence

<400> 26

Met Asp Asp Asp Asp Ser Lys Gly Glu Glu Leu Phe Thr Gly Val

Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe 20 . 25 30

Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr 35 40 45

Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr 50 55 60

Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 65 70 75 80

Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly 85 90 95

Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 100 105 110

Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile 115 120 125

Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His

Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 145 150 155 160

Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 165 170 175

Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro 180 185 190

Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr 195 200 205

Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val 210 220

Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 225 230 235 240

Leu Tyr Lys

<210> 27

<211> 732

<212> DNA

<213> Artificial Sequence <220> <223> Description of Artificial Sequence: Asp(GAT)5GFP <221> CDS <222> (1)..(732) <400> 27 48 atg gat gat gat gat agc aag ggc gag gaa ctg ttc act ggc gtg Met Asp Asp Asp Asp Ser Lys Gly Glu Glu Leu Phe Thr Gly Val 10 96 gtc cca att ctc gtg gaa ctg gat ggc gat gtg aat ggg cac aaa ttt Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe 144 tct gtc agc gga gag ggt gaa ggt gat gcc aca tac gga aag ctc acc Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr 192 ctg aaa ttc atc tgc acc act gga aag ctc cct gtg cca tgg cca aca Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr 55 240 ctg gtc act acc ttc tct tat ggc gtg cag tgc ttt tcc aga tac cca Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 288 gac cat atg aag cag cat gac ttt ttc aag agc gcc atg ccc gag ggc Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly tat gtg cag gag aga acc atc ttt ttc aaa gat gac ggg aac tac aag 336 Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 100 acc cgc gct gaa gtc aag ttc gaa ggt gac acc ctg gtg aat aga atc 384 Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile 115 120 gag ctg aag ggc att gac ttt aag gag gat gga aac att ctc ggc cac 432 Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His 480 aag ctg gaa tac aac tat aac tcc cac aat gtg tac atc atg gcc gac Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 150 155 528 aag caa aag aat ggc atc aag gtc aac ttc aag atc aga cac aac att Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 170 576 gag gat gga tcc gtg cag ctg gcc gac cat tat caa cag aac act cca Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro 185 180 atc ggc gac ggc cct gtg ctc ctc cca gac aac cat tac ctg tcc acc 624 Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr 672 cag tot goo otg tot aaa gat ooc aac gaa aag aga gac cac atg gto Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val

220 215. 210 ctg ctg gag ttt gtg acc gct gct ggg atc aca cat ggc atg gac gag Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 720 230 732 ctg tac aag tga Leu Tyr Lys <210> 28 <211> 243 <212> PRT <213> Artificial Sequence <400> 28 Met Asp Asp Asp Asp Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile 115 Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 165 170 Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr 195 ·

Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val

Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu

Leu Tyr Lys

<210> 29 <211> 732 <212> DNA <213> Artificial Sequence <220> <223> Description of Artificial Sequence: Cys(TGC)5GFP <220> <221> CDS <222> (1)..(732) <400> 29 atg tgc tgc tgc tgc agc aag ggc gag gaa ctg ttc act ggc gtg 48 Met Cys Cys Cys Cys Cys Ser Lys Gly Glu Glu Leu Phe Thr Gly Val gtc cca att ctc gtg gaa ctg gat ggc gat gtg aat ggg cac aaa ttt 96 Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe 20 144. tct gtc agc gga gag ggt gaa ggt gat gcc aca tac gga aag ctc acc Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr 192 ctg aaa ttc atc tgc acc act gga aag ctc cct gtg cca tgg cca aca Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr 240 ctg gtc act acc ttc tct tat ggc gtg cag tgc ttt tcc aga tac cca Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro gac cat atg aag cag cat gac ttt ttc aag agc gcc atg ccc gag ggc 288 Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly 95 tat gtg cag gag aga acc atc ttt ttc aaa gat gac ggg aac tac aag 336 Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 100 384 acc cgc gct gaa gtc aag ttc gaa ggt gac acc ctg gtg aat aga atc Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile 115 gag ctg aag ggc att gac ttt aag gag gat gga aac att ctc ggc cac 432 Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His 130 aag ctg gaa tac aac tat aac tcc cac aat gtg tac atc atg gcc gac 480 Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 155 150 145 aag caa aag aat ggc atc aag gtc aac ttc aag atc aga cac aac att 528 Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 165 gag gat gga tcc gtg cag ctg gcc gac cat tat caa cag aac act cca 576 Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro 180 atc ggc gac ggc cct gtg ctc ctc cca gac aac cat tac ctg tcc acc Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr 200 195

cag Gln	tct Ser 210	gcc Ala	ctg Leu	tct Ser	aaa Lys	gat Asp 215	ccc Pro	aac Asn	gaa Glu	aag Lys	aga Arg 220	gac Asp	cac His	atg Met	gtc Val	672
ctg Leu 225	ctg Leu	gag Glu	ttt Phe	gtg Val	acc Thr 230	gct Ala	gct Ala	ggg Gly	atc Ile	aca Thr 235	cat His	ggc Gly	atg Met	gac Asp	gag Glu 240	720
_	tac Tyr		tga													732
<211 <212	0> 30 L> 24 2> PF B> Ar	13 RT	lcial	l Sec	quenc	ce										
<400 Met 1	)> 3( Cys	) Cys	Cys	Cys 5	Cys	Ser	Lys	Gly	Glu 10	Glu	Leu	Phe	Thr	Gly 15	Val	
Val	Pro	Ile	Leu 20	Val	Glu	Leu	Asp	Gly 25	Asp	Val	Asn	Gly	His 30	Lys	Phe	
Ser	Val	Ser 35	Gly	Glu	Gly	Glu	Gly 40	Asp	Ala	Thr	Tyr	Gly 45	Lys	Leu	Thr	
Leu	Lys 50	Phe	Ile	Cys	Thr	Thr 55	Gly	Lys	Leu	Pro	Val 60	Pro	Trp	Pro	Thr	
Leu 65	Val	Thr	Thr	Phe	Ser 70	Tyr	Gly	Val	Gln	Cys 75	Phe	Ser	Arg	Tyr	Pro 80	
Asp	His	Met	Lys	Gln 85	His	Asp	Phe	Phe	Lys 90	Ser	Ala	Met	Pro	Glu 95	Gly	
Tyr	Val	Gln	Glu 100	Arg	Thr	Ile	Phe	Phe 105	Lys	Asp	Asp	Gly	Asn 110	Tyr	Lys	
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Glu	Leu 130	Lys	Gly	Ile	Asp	Phe 135	Lys	Glu	Asp	Gly	Asn 140	Ile	Leu	Gly	His	
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				165					170					Asn 175		
			180					185					190	Thr		
	_	195					200					205		Ser		
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cag tct	Ala	ctg Leu	tct Ser	aaa Lys	Asp	ccc	aac Asn	gaa Glu	aag Lys	Arg	gac	cac His	atg Met	gtc Val	672
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Tyr Val	. Gln	Glu 100	Arg	Thr	Ile	Phe	Phe 105	Lys	Asp	Asp	Gly	Asn 110	Tyr	Lys	
Thr Arc	y Ala 115	Glu	Val	Lys	Phe	Glu 120	Gly	Asp	Thr	Leu	Val 125	Asn	Arg	Ile	
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Lys Let 145	ı Glu	Tyr	Asn	Tyr 150	Asn	Ser	His	Asn	Val 155	Tyr	Ile	Met	Ala	Asp 160	
Lys Glr	Lys	Asn	Gly 165	Ile	Lys	Val	Asn	Phe 170	Lys	Ile	Arg	His	Asn 175	Ile	
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Gln Ser 210		Leu	Ser	Lys	Asp 215	Pro	Asn	Glu	Lys	Arg 220	Asp	His	Met	Val	

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ctg Leu 225	ctg Leu	gag Glu	ttt Phe	gtg Val	acc Thr 230	gct Ala	gct Ala	ggg Gly	atc Ile	aca Thr 235	cat His	ggc Gly	atg Met	gac Asp	gag Glu 240	720
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Ile	Ģly	Asp 195	Gly	Pro	Val	Leu	Leu 200	Pro	Asp	Asn	His	Tyr 205	Leu	Ser	Thr	

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Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile

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	n His Asp Phe Ph	ne Lys Ser Ala Met	Pro Glu Gly
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Tyr Val Gln Glu Ar 100	g Thr Ile Phe Ph	ne Lys Asp Asp Gly	Asn Tyr Lys 110
Thr Arg Ala Glu Va	l Lys Phe Glu Gl	ly Asp Thr Leu Val	Asn Arg Ile
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Lys Leu Glu Tyr As	n Tyr Asn Ser Hi	is Asn Val Tyr Ile	Met Ala Asp
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Lys Gln Lys Asn Gl		on Phe Lys Ile Arg	His Asn Ile
16		170	175
Glu Asp Gly Ser Va	l Gln Leu Ala As	sp His Tyr Gln Gln	Asn Thr Pro
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Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr 205 Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val 215 210 Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys <210> 37 <211> 732 <212> DNA <213> Artificial Sequence <220> <223> Description of Artificial Sequence: Glu(GAA)5GFP <221> CDS <222> (1)..(732) <400> 37 atg gaa gaa gaa gaa agc aag ggc gag gaa ctg ttc act ggc gtg Met Glu Glu Glu Glu Ser Lys Gly Glu Glu Leu Phe Thr Gly Val 48 qtc cca att ctc gtg gaa ctg gat ggc gat gtg aat ggg cac aaa ttt Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe tct gtc agc gga gag ggt gaa ggt gat gcc aca tac gga aag ctc acc 144 Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr ctg aaa ttc atc tgc acc act gga aag ctc cct gtg cca tgg cca aca Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr 192 55 ctg gtc act acc ttc tct tat ggc gtg cag tgc ttt tcc aga tac cca 240 Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 288 gac cat atg aag cag cat gac ttt ttc aag agc gcc atg ccc gag ggc Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly 90 85 tat gtg cag gag aga acc atc ttt ttc aaa gat gac ggg aac tac aag 336 Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 110 100 384 acc cgc gct gaa gtc aag ttc gaa ggt gac acc ctg gtg aat aga atc Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile 125 120 115 gag ctg aag ggc att gac ttt aag gag gat gga aac att ctc ggc cac 432 Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His 130 aag ctg gaa tac aac tat aac tcc cac aat gtg tac atc atg gcc gac Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 480

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Lvs							0	,,,,	70	17 - 7	m	т1.	Mot	712	Λcn	
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Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 235 Leu Tyr Lys <210> 41 <211> 732 <212> DNA <213> Artificial Sequence `` <223> Description of Artificial Sequence: Gly(GGA)5GFP <220> <221> CDS <222> (1)..(732) <400> 41 atg gga gga gga gga agc aag ggc gag gaa ctg ttc act ggc gtg Met Gly Gly Gly Gly Ser Lys Gly Glu Glu Leu Phe Thr Gly Val gtc cca att ctc gtg gaa ctg gat ggc gat gtg aat ggg cac aaa ttt 96 Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe tet gte age gga gag ggt gaa ggt gat gee aca tae gga aag ete ace 144 Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr ctg aaa ttc atc tgc acc act gga aag ctc cct gtg cca tgg cca aca 192 Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr ctg gtc act acc ttc tct tat ggc gtg cag tgc ttt tcc aga tac cca 240 Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 65 gac cat atg aag cag cat gac ttt ttc aag agc gcc atg ccc gag ggc 288 Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly tat gtg cag gag aga acc atc ttt ttc aaa gat gac ggg aac tac aag 336 Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 105 100 acc cgc gct gaa gtc aag ttc gaa ggt gac acc ctg gtg aat aga atc Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile 120 432 gag ctg aag ggc att gac ttt aag gag gat gga aac att ctc ggc cac

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aag Lys	caa Gln	aag Lys	aat Asn	ggc Gly 165	atc	aag Lys	gtc Val	aac Asn	ttc Phe 170	aag Lys	atc Ile	aga Arg	cac His	aac Asn 175	att Ile	528
gag Glu	gat Asp	gga Gly	tcc Ser 180	gtg Val	cag Gln	ctg Leu	gcc Ala	gac Asp 185	cat His	tat Tyr	caa Gln	cag Gln	aac Asn 190	act Thr	cca Pro	576
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ctg Leu 225	ctg Leu	gag Glu	ttt Phe	gtg Val	acc Thr 230	gct Ala	gct Ala	ggg Gly	atc Ile	aca Thr 235	cat His	ggc Gly	atg Met	gac Asp	gag Glu 240	720
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Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp . 150 145 Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro 180 Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 235 230 Leu Tyr Lys <210> 43 <211> 732 <212> DNA <213> Artificial Sequence <220> <223> Description of Artificial Sequence: Gly(GGC)5GFP <220> <221> CDS <222> (1) .. (732) <400> 43 atg ggc ggc ggc ggc agc aag ggc gag gaa ctg ttc act ggc gtg 48 Met Gly Gly Gly Gly Ser Lys Gly Glu Glu Leu Phe Thr Gly Val 96 gtc cca att ctc gtg gaa ctg gat ggc gat gtg aat ggg cac aaa ttt Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe 25 144 tct gtc agc gga gag ggt gaa ggt gat gcc aca tac gga aag ctc acc Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr 192 ctg aaa ttc atc tgc acc act gga aag ctc cct gtg cca tgg cca aca Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr 240 ctg gtc act acc ttc tct tat ggc gtg cag tgc ttt tcc aga tac cca Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 288 gac cat atg aag cag cat gac ttt ttc aag agc gcc atg ccc gag ggc Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly 90 tat gtg cag gag aga acc atc ttt ttc aaa gat gac ggg aac tac aag 336 Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 105 100 384 acc cgc gct gaa gtc aag ttc gaa ggt gac acc ctg gtg aat aga atc Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile

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aag ctg gaa Lys Leu Glu 145	tac aac Tyr Asn	tat a Tyr A 150	aac tc Asn Se	c cac r His	aat Asn	gtg Val 155	tac Tyr	atc Ile	atg Met	gcc Ala	gac Asp 160	480
aag caa aag Lys Gln Lys	aat ggc Asn Gly 165	atc a Ile I	aag gt Lys Va	c aac l Asn	ttc Phe 170	aag Lys	atc Ile	aga Arg	cac His	aac Asn 175	att Ile	528
gag gat gga Glu Asp Gly	tcc gtg Ser Val 180	cag c Gln I	ctg gc Leu Al	c gac a Asp 185	cat His	tat Tyr	caa Gln	cag Gln	aac Asn 190	act Thr	cca Pro	576
atc ggc gac Ile Gly Asp 195	ggc cct Gly Pro	gtg c Val I	ctc ct Leu Le 20	u Pro	gac Asp	aac Asn	cat His	tac Tyr 205	ctg Leu	tcc Ser	acc Thr	624
cag tct gcc Gln Ser Ala 210	ctg tct Leu Ser	Lys P	gat cc Asp Pr 215	c aac o Asn	gaa Glu	aag Lys	aga Arg 220	gac Asp	cac His	atg Met	gtc Val	672
ctg ctg gag Leu Leu Glu 225	ttt gtg Phe Val	acc of Thr A 230	gct gc Ala Al	t ggg a Gly	atc Ile	aca Thr 235	cat His	ggc Gly	atg Met	gac Asp	gag Glu 240	720
ctq tac aag	tga											732
Leu Tyr Lys	- <b>J</b> -											
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Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His 135 Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 145 Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro 185 Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val 220 Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys <210> 45 <211> 732 <212> DNA <213> Artificial Sequence <223> Description of Artificial Sequence: Gly(GGG)5GFP <220> <221> CDS <222> (1) ... (732) <400> 45 atg ggg ggg ggg ggg agc aag ggc gag gaa ctg ttc act ggc gtg Met Gly Gly Gly Gly Ser Lys Gly Glu Glu Leu Phe Thr Gly Val 48 96 gtc cca att ctc gtg gaa ctg gat ggc gat gtg aat ggg cac aaa ttt Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe tct gtc agc gga gag ggt gaa ggt gat gcc aca tac gga aag ctc acc Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr 35 192 ctg aaa ttc atc tgc acc act gga aag ctc cct gtg cca tgg cca aca Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr 50 240 ctg gtc act acc ttc tct tat ggc gtg cag tgc ttt tcc aga tac cca Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 70 65 gac cat atg aag cag cat gac ttt ttc aag agc gcc atg ccc gag ggc 288 Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly tat gtg cag gag aga acc atc ttt ttc aaa gat gac ggg aac tac aag 336 Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 105 110 100

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Leu	Lys 50	Phe	Ile	Cys	Thr	Thr 55	Gly	Lys	Leu	Pro	Val 60	Pro	Trp	Pro	Thr	
Leu 65	Val	Thr	Thr	Phe	Ser 70	Tyr	Gly	Val	Gln	Cys 75	Phe	Ser	Arg	Tyr	Pro 80	
Asp	His	Met	Lys	Gln 85	His	Asp	Phe	Phe	Lys 90	Ser	Ala	Met	Pro	Glu 95	Gly	
Tyr	Val	Gln	Glu 100	Arg	Thr	Ile	Phe	Phe 105	Lys	Asp	Asp	Gly	Asn 110	Tyr	Lys	

Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile 120 Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His 130 Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 170 Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr 200 Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val 215 Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 230 Leu Tyr Lys <210> 47 <211> 732 <212> DNA <213> Artificial Sequence <223> Description of Artificial Sequence: Gly(GGT)5GFP <221> CDS <222> (1)..(732) <400> 47 48 atg ggt ggt ggt ggt agc aag ggc gag gaa ctg ttc act ggc gtg Met Gly Gly Gly Gly Ser Lys Gly Glu Glu Leu Phe Thr Gly Val gtc cca att ctc gtg gaa ctg gat ggc gat gtg aat ggg cac aaa ttt 96 Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe 20 tet gtc agc gga gag ggt gaa ggt gat gcc aca tac gga aag etc acc 144 Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr ctg aaa ttc atc tgc acc act gga aag ctc cct gtg cca tgg cca aca Leu Lys Phe`Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr 192 55 ctg gtc act acc ttc tct tat ggc gtg cag tgc ttt tcc aga tac cca 240 Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 288 gac cat atg aag cag cat gac ttt ttc aag agc gcc atg ccc gag ggc Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly

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gag c Glu I 1	ctg Leu L30	aag Lys	ggc Gly	att Ile	gac Asp	ttt Phe 135	aag Lys	gag Glu	gat Asp	gga Gly	aac Asn 140	att Ile	ctc Leu	ggc Gly	cac His	432
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atc g Ile G	ggc Sly	gac Asp 195	ggc Gly	cct Pro	gtg Val	ctc Leu	ctc Leu 200	cca Pro	gac Asp	aac Asn	cat His	tac Tyr 205	ctg Leu	tcc Ser	acc Thr	624
cag t Gln S	ect Ser 210	gcc Ala	ctg Leu	tct Ser	aaa Lys	gat Asp 215	ccc Pro	aac Asn	gaa Glu	aag Lys	aga Arg 220	gac Asp	cac His	atg Met	gtc Val	672
ctg c ·Leu I 225	ctg Leu	gag Glu	ttt Phe	gtg Val	acc Thr 230	gct Ala	gct Ala	ggg Gly	atc Ile	aca Thr 235	cat His	ggc Gly	atg Met	gac Asp	gag Glu 240	720
ctg t Leu T	1	_	tga													732
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Ser V	√al	Ser 35	Gly	Glu	G1y	Glu	Gly 40	Asp	Ala	Thr	Tyr	Gly 45	Lys	Leu	Thr	
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Leu V 65	Val	Thr	Thr	Phe	Ser 70	Tyr	Gly	Val	Gln	Cys 75	Phe	Ser	Arg	Tyr	Pro 80	
Asp H	His	Met	Lys	Gln 85	His	Asp	Phe	Phe	Lys 90	Ser	Ala	Met	Pro	Glu 95	Gly	

Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 100 Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 150 Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 170 Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala' Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val 215 Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 230 Leu Tyr Lys <210> 49 <211> 732 <212> DNA <213> Artificial Sequence <220> <223> Description of Artificial Sequence: His(CAC)5GFP <220> <221> CDS <222> (1)..(732) <400> 49 48 atg cac cac cac cac agc aag ggc gag gaa ctg ttc act ggc gtg Met His His His His Ser Lys Gly Glu Glu Leu Phe Thr Gly Val 96 gtc cca att ctc gtg gaa ctg gat ggc gat gtg aat ggg cac aaa ttt Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe 20 tct gtc agc gga gag ggt gaa ggt gat gcc aca tac gga aag ctc acc 144 Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr 35 192 ctg aaa ttc atc tgc acc act gga aag ctc cct gtg cca tgg cca aca Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr 240 ctg gtc act acc ttc tct tat ggc gtg cag tgc ttt tcc aga tac cca Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro gac cat atg aag cag cat gac ttt ttc aag agc gcc atg ccc gag ggc 288

	17.2 <b>-</b>	Mat	T	Cln	uic	Aen	Phe	Phe	Lvs	Ser	Ala	Met	Pro	Glu	Gly	
Asp	ніѕ	мес	гуѕ	.85	птэ	изр	The	1110	90	-				95	-	
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aag Lys 145	ctg Leu	gaa Glu	tac Tyr	aac Asn	tat Tyr 150	aac Asn	tcc Ser	cac His	aat Asn	gtg Val 155	tac Tyr	atc Ile	atg Met	gcc Ala	gac Asp 160	480
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ctg Leu 225	Leu	gag Glu	ttt Phe	gtg Val	acc Thr 230	gct Ala	gct Ala	ggg Gly	atc Ile	aca Thr 235	cat His	ggc Gly	atg Met	gac Asp	gag Glu 240	720
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Ser	Val	Ser 35		Glu	Gly	Glu	Gly 40		Ala	Thr	Tyr	Gly 45	Lys	Leu	Thr	
Leu	Lys 50		Ile	Cys	Thr	Thr 55		Lys	Leu	Pro	Val 60		Trp	Pro	Thr	
Leu 65		Thr	Thr	Phe	Ser 70		Gly	Val	Gln	Cys 75	Phe	Ser	Arg	Tyr	Pro 80	

Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 100 Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 150 Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro 185 Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr 200 Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 230 Leu Tyr Lys <210> 51 <211> 732 <212> DNA <213> Artificial Sequence <223> Description of Artificial Sequence: His(CAT)5GFP <220> <221> CDS <222> (1)..(732) <400> 51 atg cat cat cat cat agc aag ggc gag gaa ctg ttc act ggc gtg 48 Met His His His His Ser Lys Gly Glu Glu Leu Phe Thr Gly Val 96 gtc cca att ctc gtg gaa ctg gat ggc gat gtg aat ggg cac aaa ttt Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe tct gtc agc gga gag ggt gaa ggt gat gcc aca tac gga aag ctc acc Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr ctg aaa ttc atc tgc acc act gga aag ctc cct gtg cca tgg cca aca 192 Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr ctg gtc act acc ttc tct tat ggc gtg cag tgc ttt tcc aga tac cca 240 Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro

65					70					75					80	
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tat Tyr	gtg Val	cag Gln	gag Glu 100	aga Arg	acc Thr	atc Ile	ttt Phe	ttc Phe 105	aaa Lys	gat Asp	gac Asp	ggg Gly	aac Asn 110	tac Tyr	aag Lys	336
acc Thr	cgc Arg	gct Ala 115	gaa Glu	gtc Val	aag Lys	ttc Phe	gaa Glu 120	ggt Gly	gac Asp	acc Thr	ctg Leu	gtg Val 125	aat Asn	aga Arg	atc Ile	384
gag Glu	ctg Leu 130	aag Lys	ggc Gly	att Ile	gac Asp	ttt Phe 135	aag Lys	gag Glu	gat Asp	gga Gly	aac Asn 140	att Ile	ctc Leu	ggc Gly	cac His	432
aag Lys 145	ctg Leu	gaa Glu	tac Tyr	aac Asn	tat Tyr 150	aac Asn	tcc Ser	cac His	aat Asn	gtg Val 155	tac Tyr	atc Ile	atg Met	gcc Ala	gac Asp 160	480
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gag Glu	gat Asp	gga Gly	tcc Ser 180	gtg Val	cag Gln	ctg Leu	gcc Ala	gac Asp 185	cat His	tat Tyr	caa Gln	cag Gln	aac Asn 190	act Thr	cca Pro	576
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cag Gln	tct Ser 210	gcc Ala	ctg Leu	tct Ser	aaa Lys	gat Asp 215	ccc Pro	aac Asn	gaa Glu	aag Lys	aga Arg 220	gac Asp	cac His	atg Met	gtc Val	672
ctg Leu 225	ctg Leu	gag Glu	ttt Phe	gtg Val	acc Thr 230	gct Ala	gct Ala	ggg Gly	atc Ile	aca Thr 235	cat His	ggc Gly	atg Met	gac Asp	gag Glu 240	720
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Ser	Val	Ser 35	Gly	Glu	Gly	Glu	Gly 40	Asp	Ala	Thr	Tyr	Gly 45	Lys	Leu	Thr	
Leu	Lys 50	Phe	Ile	Cys	Thr	Thr 55	Gly	Lys	Leu	Pro	Val 60	Pro	Trp	Pro	Thr	

Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile 120 Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 150 Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 165 Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro 185 Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr 200 Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 230 235 Leu Tyr Lys <210> 53 <211> 732 <212> DNA <213> Artificial Sequence <223> Description of Artificial Sequence: Ile(ATA)5GFP <220> <221> CDS <222> (1)..(732) <400> 53 48 atg ata ata ata ata agc aag ggc gag gaa ctg ttc act ggc gtg Met Ile Ile Ile Ile Ser Lys Gly Glu Glu Leu Phe Thr Gly Val gtc cca att ctc gtg gaa ctg gat ggc gat gtg aat ggg cac aaa ttt Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe 20 tct gtc agc gga gag ggt gaa ggt gat gcc aca tac gga aag ctc acc 144 Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr 192 ctg aaa ttc atc tgc acc act gga aag ctc cct gtg cca tgg cca aca Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr 60 55

ctg gtc Leu Val 65	act a Thr T	acc Thr	ttc Phe	tct Ser 70	tat Tyr	ggc Gly	gtg Val	cag Gln	tgc Cys 75	ttt Phe	tcc Ser	aga Arg	tac Tyr	cca Pro 80	240
gac cat Asp His	atg a Met L	aag Lys	cag Gln 85	cat His	gac Asp	ttt Phe	ttc Phe	aag Lys 90	agc Ser	gcc Ala	atg Met	ccc Pro	gag Glu 95	ggc Gly	288
tat gtg Tyr Val	Gln G	gag Slu 100	aga Arg	acc Thr	atc Ile	ttt Phe	ttc Phe 105	aaa Lys	gat Asp	gac Asp	ggg Gly	aac Asn 110	tac Tyr	aag Lys	336
acc cgc Thr Arg	gct g Ala G 115	gaa Glu	gtc Val	aag Lys	ttc Phe	gaa Glu 120	ggt Gly	gac Asp	acc Thr	ctg Leu	gtg Val 125	aat Asn	aga Arg	atc Ile	384
gag ctg Glu Leu 130	aag g Lys G	gly	att Ile	gac Asp	ttt Phe 135	aag Lys	gag Glu	gat Asp	gga Gly	aac Asn 140	att Ile	ctc Leu	ggc Gly	cac His	432
aag ctg Lys Leu 145	gaa t Glu T	cac Tyr .	aac Asn	tat Tyr 150	aac Asn	tcc Ser	cac His	aat Asn	gtg Val 155	tac Tyr	atc Ile	atg Met	gcc Ala	gac Asp 160	480
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atc ggc Ile Gly	gac g Asp G 195	ggc	cct Pro	gtg Val	ctc Leu	ctc Leu 200	cca Pro	gac Asp	aac Asn	cat His	tac Tyr 205	ctg Leu	tcc Ser	acc Thr	624
cag tct Gln Ser 210	Ala I	ctg Leu	tct Ser	aaa Lys	gat Asp 215	ccc Pro	aac Asn	gaa Glu	aag Lys	aga Arg 220	gac Asp	cac His	atg Met	gtc Val	672
ctg ctg Leu Leu 225	gag t Glu F	tt Phe	gtg Val	acc Thr 230	gct Ala	gct Ala	ggg Gly	atc Ile	aca Thr 235	cat His	ggc Gly	atg Met	gac Asp	gag Glu 240	720
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Ser Val	Ser G	Gly	Glu	Gly	Glu	Gly 40	Asp	Ala	Thr	Tyr	Gly 45	Lys	Leu	Thr	

Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 105 Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His 135 Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys <210> 55 <211> 732 <212> DNA <213> Artificial Sequence <223> Description of Artificial Sequence: Ile(ATC)5GFP <220> <221> CDS <222> (1)..(732) <400> 55 48 atg atc atc atc atc agc aag ggc gag gaa ctg ttc act ggc gtg Met Ile Ile Ile Ile Ile Ser Lys Gly Glu Glu Leu Phe Thr Gly Val 10 gtc cca att ctc gtg gaa ctg gat ggc gat gtg aat ggg cac aaa ttt 96 Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe tct gtc agc gga gag ggt gaa ggt gat gcc aca tac gga aag ctc acc 144 Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr

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ctg Leu 65	gtc Val	act Thr	acc Thr	ttc Phe	tct Ser 70	tat Tyr	ggc Gly	gtg Val	cag Gln	tgc Cys 75	ttt Phe	tcc Ser	aga Arg	tac Tyr	cca Pro 80	240
gac Asp	cat His	atg Met	aag Lys	cag Gln 85	cat His	gac Asp	ttt Phe	ttc Phe	aag Lys 90	agc Ser	gcc Ala	atg Met	ccc Pro	gag Glu 95	ggc Gly	288
tat Tyr	gtg Val	cag Gln	gag Glu 100	aga Arg	acc Thr	atc Ile	ttt Phe	ttc Phe 105	aaa Lys	gat Asp	gac Asp	ggg Gly	aac Asn 110	tac Tyr	aag Lys	336
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aag Lys 145	ctg Leu	gaa Glu	tac Tyr	aac Asn	tat Tyr 150	aac Asn	tcc Ser	cac His	aat Asn	gtg Val 155	tac Tyr	atc Ile	atg Met	gcc Ala	gac Asp 160	480
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atc Ile	ggc Gly	gac Asp 195	ggc Gly	cct Pro	gtg Val	ctc Leu	ctc Leu 200	cca Pro	gac Asp	aac Asn	cat His	tac Tyr 205	ctg Leu	tcc Ser	acc Thr	624
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ctg Leu 225	Leu	gag Glu	ttt Phe	gtg Val	acc Thr 230	gct Ala	gct Ala	ggg Gly	atc Ile	aca Thr 235	cat His	ggc Gly	atg Met	gac Asp	gag Glu 240	720
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Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr 40 Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile 120 Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His 130 135 Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 150 Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 170 Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr 200 Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val 210 Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 230 235 Leu Tyr Lys <210> 57 <211> 732 <212> DNA <213> Artificial Sequence <223> Description of Artificial Sequence: Ile(ATT)5GFP <220> <221> CDS <222> (1)..(732) <400> 57 atg att att att att agc aag ggc gag gaa ctg ttc act ggc gtg 48 Met Ile Ile Ile Ile Ser Lys Gly Glu Glu Leu Phe Thr Gly Val gtc cca att ctc gtg gaa ctg gat ggc gat gtg aat ggg cac aaa ttt Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe

tct gtc agc gga gag ggt gaa ggt gat gcc aca tac gga aag ctc acc

S	er	Val	Ser 35	Gly	Glu	Gly	Glu	Gly 40	Asp	Ala	Thr	Tyr	Gly 45	Lys	Leu	Thr	
C	tg Leu	aaa Lys 50	ttc Phe	atc Ile	tgc Cys	acc Thr	act Thr 55	gga Gly	aag Lys	ctc Leu	cct Pro	gtg Val 60	cca Pro	tgg Trp	cca Pro	aca Thr	192
C	etg Leu 65	gtc Val	act Thr	acc Thr	ttc Phe	tct Ser 70	tat Tyr	ggc Gly	gtg Val	cag Gln	tgc Cys 75	ttt Phe	tcc Ser	aga Arg	tac Tyr	cca Pro 80	240
Ç	gac Asp	cat His	atg Met	aag Lys	cag Gln 85	cat His	gac Asp	ttt Phe	ttc Phe	aag Lys 90	agc Ser	gcc Ala	atg Met	ccc Pro	gag Glu 95	ggc Gly	288
t	at Yyr	gtg Val	cag Gln	gag Glu 100	aga Arg	acc Thr	atc Ile	ttt Phe	ttc Phe 105	aaa Lys	gat Asp	gac Asp	ggg Gly	aac Asn 110	tac Tyr	aag Lys	336
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Ċ	gag Slu	ctg Leu 130	aag Lys	ggc Gly	att Ile	gac Asp	ttt Phe 135	aag Lys	gag Glu	gat Asp	gga Gly	aac Asn 140	att Ile	ctc Leu	ggc Gly	cac His	432
1	aag Lys 145	ctg Leu	gaa Glu	tac Tyr	aac Asn	tat Tyr 150	aac Asn	tcc Ser	cac His	aat Asn	gtg Val 155	tac Tyr	atc Ile	atg Met	gcc Ala	gac Asp 160	480
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	ctg Leu 225	ctg Leu	gag Glu	ttt Phe	gtg Val	acc Thr 230	Ala	gct Ala	ggg	atc Ile	aca Thr 235	His	ggc	atg Met	gac Asp	gag Glu 240	720
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Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe

30 20 25

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ctg Leu	aaa Lys 50	ttc Phe	atc Ile	tgc Cys	acc Thr	act Thr 55	gga Gly	aag Lys	ctc Leu	cct Pro	gtg Val 60	cca Pro	tgg Trp	cca Pro	aca Thr	192
ctg Leu 65	gtc Val	act Thr	acc Thr	ttc Phe	tct Ser 70	tat Tyr	ggc Gly	gtg Val	cag Gln	tgc Cys 75	ttt Phe	tcc Ser	aga Arg	tac Tyr	cca Pro 80	240
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tat Tyr	gtg Val	cag Gln	gag Glu 100	aga Arg	acc Thr	atc Ile	ttt Phe	ttc Phe 105	aaa Lys	gat Asp	gac Asp	ggg Gly	aac Asn 110	tac Tyr	aag Lys	336
acc Thr	cgc Arg	gct Ala 115	gaa Glu	gtc Val	aag Lys	ttc Phe	gaa Glu 120	ggt Gly	gac Asp	acc Thr	ctg Leu	gtg Val 125	aat Asn	aga Arg	atc Ile	384
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gag Glu	gat Asp	gga Gly	tcc Ser 180	gtg Val	cag Gln	ctg Leu	gcc Ala	gac Asp 185	cat His	tat Tyr	caa Gln	cag Gln	aac Asn 190	act Thr	cca Pro	576
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ctg Leu 225	ctg Leu	gag Glu	ttt Phe	gtg Val	acc Thr 230	gct Ala	gct Ala	ggg	atc Ile	aca Thr 235	cat His	ggc Gly	atg Met	gac Asp	gag Glu 240	720
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<212> PRT <213> Artificial Sequence

<400> 60

Met Leu Leu Leu Leu Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 100 Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile 120 Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 170 Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val 210 Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys <210> 61 <211> 732 <212> DNA <213> Artificial Sequence <223> Description of Artificial Sequence: Leu(CTC)5GFP <220> <221> CDS <222> (1)..(732) <400> 61

atg ctc ctc ctc ctc agc aag ggc gag gaa ctg ttc act ggc gtg Met Leu Leu Leu Leu Ser Lys Gly Glu Glu Leu Phe Thr Gly Val

gtc Val	cca Pro	att Ile	ctc Leu 20	gtg Val	gaa Glu	ctg Leu	gat Asp	ggc Gly 25	gat Asp	gtg Val	aat Asn	ggg Gly	cac His 30	aaa Lys	ttt Phe	96
tct Ser	gtc Val	agc Ser 35	gga Gly	gag Glu	ggt Gly	gaa Glu	ggt Gly 40	gat Asp	gcc Ala	aca Thr	tac Tyr	gga Gly 45	aag Lys	ctc Leu	acc Thr	144
ctg Leu	aaa Lys 50	ttc Phe	atc Ile	tgc Cys	acc Thr	act Thr 55	gga Gly	aag Lys	ctc Leu	cct Pro	gtg Val 60	cca Pro	tgg Trp	cca Pro	aca Thr	192
ctg Leu 65	gtc Val	act Thr	acc Thr	ttc Phe	tct Ser 70	tat Tyr	ggc Gly	gtg Val	cag Gln	tgc Cys 75	ttt Phe	tcc Ser	aga Arg	tac Tyr	cca Pro 80	240
gac Asp	cat His	atg Met	aag Lys	cag Gln 85	cat His	gac Asp	ttt Phe	ttc Phe	aag Lys 90	agc Ser	gcc Ala	atg Met	ccc Pro	gag Glu 95	Gly ggc	288
tat Tyr	gtg Val	cag Gln	gag Glu 100	aga Arg	acc Thr	atc Ile	ttt Phe	ttc Phe 105	aaa Lys	gat Asp	gac Asp	ggg Gly	aac Asn 110	tac Tyr	aag Lys	336
acc Thr	cgc Arg	gct Ala 115	gaa Glu	gtc Val	aag Lys	ttc Phe	gaa Glu 120	ggt Gly	gac Asp	acc Thr	ctg Leu	gtg Val 125	aat Asn	aga Arg	atc Ile	384
gag Glu	ctg Leu 130	aag Lys	ggc Gly	att Ile	gac Asp	ttt Phe 135	aag Lys	gag Glu	gat Asp	gga Gly	aac Asn 140	att Ile	ctc Leu	ggc Gly	cac His	432
aag Lys 145	ctg Leu	gaa Glu	tac Tyr	aac Asn	tat Tyr 150	aac Asn	tcc Ser	cac His	aat Asn	gtg Val 155	tac Tyr	atc Ile	atg Met	gcc Ala	gac Asp 160	480
aag Lys	caa Gln	aag Lys	aat Asn	ggc Gly 165	atc Ile	aag Lys	gtc Val	aac Asn	ttc Phe 170	aag Lys	atc Ile	aga Arg	cac His	aac Asn 175	att Ile	528
gag Glu	gat Asp	gga Gly	tcc Ser 180	gtg Val	cag Gln	ctg Leu	gcc Ala	gac Asp 185	cat His	tat Tyr	caa Gln	cag Gln	aac Asn 190	act Thr	cca Pro	576
atc Ile	ggc Gly	gac Asp 195	ggc Gly	cct Pro	gtg Val	ctc Leu	ctc Leu 200	cca Pro	gac Asp	aac Asn	cat His	tac Tyr 205	ctg Leu	tcc Ser	acc Thr	624
cag Gln	tct Ser 210	gcc Ala	ctg Leu	tct Ser	aaa Lys	gat Asp 215	ccc Pro	aac Asn	gaa Glu	aag Lys	aga Arg 220	gac Asp	cac His	atg Met	gtc Val	672
ctg Leu 225	ctg Leu	gag Glu	ttt Phe	gtg Val	acc Thr 230	gct Ala	gct Ala	ggg Gly	atc Ile	aca Thr 235	cat His	ggc Gly	atg Met	gac Asp	gag Glu 240	720
_	tac Tyr	_	tga													732

<210> 62 <211> 243 <212> PRT

<213> Artificial Sequence

<400> 62
Met Leu Leu Leu Leu Ser Lys Gly Glu Glu Leu Phe Thr Gly Val
1 5 10 15

Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe 20 25 30

Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr 35 40 45

Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr 50 55 60

Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 65 70 75 80

Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly 85 90 95

Tyr Val Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 100 105 110

Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile 115 120 125

Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His 130 135 140

Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 145 150 155 160

Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 165 170 175

Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro 180 185 190

Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr 195 200 205

Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val 210 215 220

Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 225 230 235 240

Leu Tyr Lys

<210> 63

<211> 732

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Leu(CTG)5GFP

<220>

<221> CDS

<222> (1)..(732)

<400> 63

i	atg Met 1	ctg Leu	ctg Leu	ctg Leu	ctg Ļeu 5	ctg Leu	agc Ser	aag Lys	ggc Gly	gag Glu 10	gaa Glu	ctg Leu	ttc Phe	act Thr	ggc Gly 15	gtg Val	48
,	gtc Val	cca Pro	att Ile	ctc Leu 20	gtg Val	gaa Glu	ctg Leu	gat Asp	ggc Gly 25	gat Asp	gtg Val	aat Asn	ggg Gly	cac His 30	aaa Lys	ttt Phe	96
;	tct Ser	gtc Val	agc Ser 35	gga Gly	gag Glu	ggt Gly	gaa Glu	ggt Gly 40	gat Asp	gcc Ala	aca Thr	tac Tyr	gga Gly 45	aag Lys	ctc Leu	acc Thr	144
]	ctg Leu	aaa Lys 50	ttc Phe	atc Ile	tgc Cys	acc Thr	act Thr 55	gga Gly	aag Lys	ctc Leu	cct Pro	gtg Val 60	cca Pro	tgg Trp	cca Pro	aca Thr	192
]	ctg Leu 65	gtc Val	act Thr	acc Thr	ttc Phe	tct Ser 70	tat Tyr	ggc Gly	gtg Val	cag Gln	tgc Cys 75	ttt Phe	tcc Ser	aga Arg	tac Tyr	cca Pro 80	240
i	gac Asp	cat His	atg Met	aag Lys	cag Gln 85	cat His	gac Asp	ttt Phe	ttc Phe	aag Lys 90	agc Ser	gcc Ala	atg Met	ccc Pro	gag Glu 95	ggc Gly	288
•	tat Tyr	gtg Val	cag Gln	gag Glu 100	aga Arg	acc Thr	atc Ile	ttt Phe	ttc Phe 105	aaa Lys	gat Asp	gac Asp	ggg Gly	aac Asn 110	tac Tyr	aag Lys	336
•	acc Thr	cgc Arg	gct Ala 115	gaa Glu	gtc Val	aag Lys	ttc Phe	gaa Glu 120	ggt Gly	gac Asp	acc Thr	ctg Leu	gtg Val 125	aat Asn	aga Arg	atc Ile	384
(	gag Glu	ctg Leu 130	aag Lys	ggc Gly	att Ile	gac Asp	ttt Phe 135	aag Lys	gag Glu	gat Asp	gga Gly	aac Asn 140	att Ile	ctc Leu	ggc Gly	cac His	432
]	aag Lys 145	ctg Leu	gaa Glu	tac Tyr	aac Asn	tat Tyr 150	aac Asn	tcc Ser	cac His	aat Asn	gtg Val 155	tac Tyr	atc Ile	atg Met	gcc Ala	gac Asp 160	480
]	aag Lys	caa Gln	aag Lys	aat Asn	ggc Gly 165	atc Ile	aag Lys	gtc Val	aac Asn	ttc Phe 170	aag Lys	atc Ile	aga Arg	cac His	aac Asn 175	att Ile	528
(	gag Glu	gat Asp	gga Gly	tcc Ser 180	gtg Val	cag Gln	ctġ Leu	gcc Ala	gac Asp 185	cat His	tat Tyr	caa Gln	cag Gln	aac Asn 190	act Thr	cca Pro	576
	atc Ile	ggc Gly	gac Asp 195	ggc Gly	cct Pro	gtg Val	ctc Leu	ctc Leu 200	cca Pro	gac Asp	aac Asn	cat His	tac Tyr 205	ctg Leu	tcc Ser	acc Thr	624
(	cag Gln	tct Ser 210	gcc Ala	ctg Leu	tct Ser	aaa Lys	gat Asp 215	ccc Pro	aac Asn	gaa Glu	aag Lys	aga Arg 220	gac Asp	cac His	atg Met	gtc Val	672
	ctg Leu 225	ctg Leu	gag Glu	ttt Phe	gtg Val	acc Thr 230	gct Ala	gct Ala	ggg Gly	atc Ile	aca Thr 235	cat His	ggc Gly	atg Met	gac Asp	gag Glu 240	720
	_	tac Tyr	_	tga													732

<210> 64 <211> 243 <212> PRT <213> Artificial Sequence

Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr 35 40 45

Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr 50 55 60

Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 65 70 75 80

Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly 85 90 95

Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 100 105 110

Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile 115 120 125

Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His 130 135 140

Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 145 150 155 160

Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 165 170 175

Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro 180 185 190

Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr 195 200 205

Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val 210 215 220

Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 225 230 235 240

Leu Tyr Lys

<210> 65

<211> 732

<212> DNA

<213> Artificial Sequence

(220>

<223> Description of Artificial Sequence: Leu(CTT)5GFP

<220> <221> CDS <222> (1)..(732)

at o	)> 65 ctt Leu	ctt	ctt Leu	ctt Leu 5	ctt Leu	agc Ser	aag Lys	ggc Gly	gag Glu 10	gaa Glu	ctg Leu	ttc Phe	act Thr	ggc Gly 15	gtg Val	48
gtc Val	cca Pro	att Ile	ctc Leu 20	gtg Val	gaa Glu	ctg Leu	gat Asp	ggc Gly 25	gat Asp	gtg Val	aat Asn	ggg Gly	cac His 30	aaa Lys	ttt Phe	96
tct Ser	gtc Val	agc Ser 35	gga Gly	gag Glu	ggt Gly	gaa Glu	ggt Gly 40	gat Asp	gcc Ala	aca Thr	tac Tyr	gga Gly 45	aag Lys	ctc Leu	acc Thr	144
ctg Leu	aaa Lys 50	ttc Phe	atc Ile	tgc Cys	acc Thr	act Thr 55	gga Gly	aag Lys	ctc Leu	cct Pro	gtg Val 60	cca Pro	tgg Trp	cca Pro	aca Thr	192
ctg Leu 65	gtc Val	act Thr	acc Thr	ttc Phe	tct Ser 70	tat Tyr	ggc Gly	gtg Val	cag Gln	tgc Cys 75	ttt Phe	tcc Ser	aga Arg	tac Tyr	cca Pro 80	240
gac Asp	cat His	atg Met	aag Lys	cag Gln 85	cat His	gac Asp	ttt Phe	ttc Phe	aag Lys 90	agc Ser	gcc Ala	atg Met	ccc Pro	gag Glu 95	ggc Gly	288
tat Tyr	gtg Val	.cag Gln	gag Glu 100	aga Arg	acc Thr	atc Ile	ttt Phe	ttc Phe 105	aaa Lys	gat Asp	gac Asp	ggg Gly	aac Asn 110	tac Tyr	aag Lys	336
acc Thr	cgc Arg	gct Ala 115	gaa Glu	gtc Val	aag Lys	ttc Phe	gaa Glu 120	ggt Gly	gac Asp	acc Thr	ctg Leu	gtg Val 125	aat Asn	aga Arg	atc Ile	384
gag Glu	ctg Leu 130	aag Lys	ggc Gly	att Ile	gac Asp	ttt Phe 135	aag Lys	gag Glu	gat Asp	gga Gly	aac Asn 140	att Ile	ctc Leu	ggc Gly	cac His	432
aag Lys 145	ctg Leu	gaa Glu	tac Tyr	aac Asn	tat Tyr 150	aac Asn	tcc Ser	cac His	aat Asn	gtg Val 155	tac Tyr	atc Ile	atg Met	gcc Ala	gac Asp 160	480
aag Lys	caa Gln	aag Lys	aat Asn	ggc Gly 165	atc Ile	aag Lys	gtc Val	aac Asn	ttc Phe 170	aag Lys	atc Ile	aga Arg	cac His	aac Asn 175	att Ile	528
gag Glu	gat Asp	gga Gly	tcc Ser 180	gtg Val	cag Gln	ctg Leu	gcc Ala	gac Asp 185	cat His	tat Tyr	caa Gln	cag Gln	aac Asn 190	act Thr	cca Pro	576
atc Ile	ggc Gly	gac Asp 195	ggc Gly	cct Pro	gtg Val	ctc Leu	ctc Leu 200	cca Pro	gac Asp	aac Asn	cat His	tac Tyr 205	ctg Leu	tcc Ser	acc Thr	624
cag Gln	tct Ser 210	gcc Ala	ctg Leu	tct Ser	aaa Lys	gat Asp 215	ccc Pro	aac Asn	gaa Glu	aag Lys	aga Arg 220	gac Asp	cac His	atg Met	gtc Val	672
ctg Leu 225	ctg Leu	gag Glu	ttt Phe	gtg Val	acc Thr 230	gct Ala	gct Ala	ggg Gly	atc Ile	aca Thr 235	cat His	ggc Gly	atg Met	gac Asp	gag Glu 240	720
ctg	tac	aag	tga													732

Leu Tyr Lys

<210> 66

<211> 243

<212> PRT

<213> Artificial Sequence

<400> 66

Met Leu Leu Leu Leu Ser Lys Gly Glu Glu Leu Phe Thr Gly Val 1 5 10 15

Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe 20 25 30

Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr 35 40 45

Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr 50 55 60

Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 65 70 75 80

Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly 85 90 95

Tyr Val Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 100 105 110

Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile 115 120 125

Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His 130 135 140

Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 145 150 155 160

Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 165 170 175

Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro 180 185 190

Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr 195 200 205

Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val 210 215 220

Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 225 230 235 240

Leu Tyr Lys

<210> 67

<211> 732

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Leu(TTA)5GFP

<220> <221> CDS <222> (1)..(732)

ato	)> 67 tta Leu	tta	tta Leu	tta Leu 5	tta Leu	agc Ser	aag Lys	ggc Gly	gag Glu 10	gaa Glu	ctg Leu	ttc Phe	act Thr	ggc Gly 15	gtg Val	48
gtc Val	cca Pro	att Ile	ctc Leu 20	gtg Val	gaa Glu	ctg Leu	gat Asp	ggc Gly 25	gat Asp	gtg Val	aat Asn	Gly ggg	cac His 30	aaa Lys	ttt Phe	96
tct Ser	gtc Val	agc Ser 35	gga Gly	gag Glu	ggt Gly	gaa Glu	ggt Gly 40	gat Asp	gcc Ala	aca Thr	tac Tyr	gga Gly 45	aag Lys	ctc Leu	acc Thr	144
ctg Leu	aaa Lys 50	ttc Phe	atc Ile	tgc Cys	acc Thr	act Thr 55	gga Gly	aag Lys	ctc Leu	cct Pro	gtg Val 60	cca Pro	tgg Trp	cca Pro	aca Thr	192
ctg Leu 65	gtc Val	act Thr	acc Thr	ttc Phe	tct Ser 70	tat Tyr	ggc Gly	gtg Val	cag Gln	tgc Cys 75	ttt Phe	tcc Ser	aga Arg	tac Tyr	cca Pro 80	240
gac Asp	cat His	atg Met	aag Lys	cag Gln 85	cat His	gac Asp	ttt Phe	ttc Phe	aag Lys 90	agc Ser	gcc Ala	atg Met	ccc Pro	gag Glu 95	ggc Gly	288
tat Tyr	gtg Val	cag Gln	gag Glu 100	aga Arg	acc Thr	atc Ile	ttt Phe	ttc Phe 105	aaa Lys	gat Asp	gac Asp	ggg Gly	aac Asn 110	tac Tyr	aag Lys	336
acc Thr	cgc Arg	gct Ala 115	gaa Glu	gtc Val	aag Lys	ttc Phe	gaa Glu 120	ggt Gly	gac Asp	acc Thr	ctg Leu	gtg Val 125	aat Asn	aga Arg	atc Ile	384
gag Glu	ctg Leu 130	aag Lys	ggc Gly	att Ile	gac Asp	ttt Phe 135	aag Lys	gag Glu	gat Asp	gga Gly	aac Asn 140	att Ile	ctc Leu	ggc Gly	cac His	432
aag Lys 145	ctg Leu	gaa Glu	tac Tyr	aac Asn	tat Tyr 150	aac Asn	tcc Ser	cac His	aat Asn	gtg Val 155	tac Tyr	atc Ile	atg Met	gcc Ala	gac Asp 160	480
aag Lys	caa Gln	aag Lys	aat Asn	ggc Gly 165	atc Ile	aag Lys	gtc Val	aac Asn	ttc Phe 170	aag Lys	atc Ile	aga Arg	cac His	aac Asn 175	att Ile	528
gag Glu	gat Asp	gga Gly	tcc Ser 180	gtg Val	cag Gln	ctg Leu	gcc Ala	gac Asp 185	cat His	tat Tyr	caa Gln	cag Gln	aac Asn 190	act Thr	cca Pro	576
atc Ile	ggc Gly	gac Asp 195	ggc Gly	cct Pro	gtg Val	ctc Leu	ctc Leu 200	cca Pro	gac Asp	aac Asn	cat His	tac Tyr 205	ctg Leu	tcc Ser	acc Thr	624
cag Gln	tct Ser 210	gcc Ala	ctg Leu	tct Ser	aaa Lys	gat Asp 215	ccc Pro	aac Asn	gaa Glu	aag Lys	aga Arg 220	gac Asp	cac His	atg Met	gtc Val	672
ctg Leu	ctg Leu	gag Glu	ttt Phe	gtg Val	acc Thr	gct Ala	gct Ala	ggg Gly	atc Ile	aca Thr	cat His	ggc Gly	atg Met	gac Asp	gag Glu	720

732

225 230 235 240

ctg tac aag tga Leu Tyr Lys

eu Tyr Lys

<210> 68

<211> 243

<212> PRT

<213> Artificial Sequence

<400> 68

Met Leu Leu Leu Leu Ser Lys Gly Glu Glu Leu Phe Thr Gly Val 1 5 10

Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe 20 25 30

Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr 35 40 45

Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr 50 55 60

Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 65 70 75 80

Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly 85 90 95

Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys
100 105 110

Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile 115 . 120 . 125

Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His 130 135 140

Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 145 150 155 160

Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 165 170 175

Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro 180 185 190

Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr 195 200 205

Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val 210 215 220

Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 225 230 235 240

Leu Tyr Lys

<210> 69

<211> 732

<212> DNA <213> Artificial Sequence

<220 <223		escri	iptic	on of	E Art	cific	cial	Sequ	ience	e: Le	eu (T	rg) 50	GFP			
	L> CI		(732)	)							,					
ato	)> 69 ttg Leu	tta	ttg Leu	ttg Leu 5	ttg Leu	agc Ser	aag Lys	ggc Gly	gag Glu 10	gaa Glu	ctg Leu	ttc Phe	act Thr	ggc Gly 15	gtg Val	48
gtc Val	cca Pro	att Ile	ctc Leu 20	gtg Val	gaa Glu	ctg Leu	gat Asp	ggc Gly 25	gat Asp	gtg Val	aat Asn	ggg Gly	cac His 30	aaa Lys	ttt Phe	96
tct Ser	gtc Val	agc Ser 35	gga Gly	gag Glu	ggt Gly	gaa Glu	ggt Gly 40	gat Asp	gcc Ala	aca Thr	tac Tyr	gga Gly 45	aag Lys	ctc Leu	acc Thr	144
ctg Leu	aaa Lys 50	ttc Phe	atc Ile	tgc Cys	acc Thr	act Thr 55	gga Gly	aag Lys	ctc Leu	cct Pro	gtg Val 60	cca Pro	tgg Trp	cca Pro	aca Thr	192
ctg Leu 65	gtc Val	act Thr	acc Thr	ttc Phe	tct Ser 70	tat Tyr	ggc Gly	gtg Val	cag Gln	tgc Cys 75	ttt Phe	tcc Ser	aga Arg	tac Tyr	cca Pro 80	240
gac Asp	cat His	atg Met	aag Lys	cag Gln 85	cat His	gac Asp	ttt Phe	ttc Phe	aag Lys 90	agc Ser	gcc Ala	atg Met	ccc Pro	gag Glu 95	ggc Gly	288
tat Tyr	gtg Val	cag Gln	gag Glu 100	aga Arg	acc Thr	atc Ile	ttt Phe	ttc Phe 105	aaa Lys	gat Asp	gac Asp	ggg Gly	aac Asn 110	tac Tyr	aag Lys	336
acc Thr	cgc Arg	gct Ala 115	gaa Glu	gtc Val	aag Lys	ttc Phe	gaa Glu 120	ggt Gly	gac Asp	acc Thr	ctg Leu	gtg Val 125	aat Asn	aga Arg	atc Ile	384
gag Glu	ctg Leu 130	aag Lys	ggc Gly	att Ile	gac Asp	ttt Phe 135	aag Lys	gag Glu	gat Asp	gga Gly	aac Asn 140	att Ile	ctc Leu	ggc Gly	cac His	432
aag Lys 145	ctg Leu	gaa Glu	tac Tyr	aac Asn	tat Tyr 150	aac Asn	tcc Ser	cac His	aat Asn	gtg Val 155	tac Tyr	atc Ile	atg Met	gcc Ala	gac Asp 160	480
aag Lys	caa Gln	aag Lys	aat Asn	ggc Gly 165	atc Ile	aag Lys	gtc Val	aac Asn	ttc Phe 170	aag Lys	atc Ile	aga Arg	cac His	aac Asn 175	att Ile	528
gag Glu	gat Asp	gga Gly	tcc Ser 180	gtg Val	cag Gln	ctg Leu	gcc Ala	gac Asp 185	cat His	tat Tyr	caa Gln	cag Gln	aac Asn 190	act Thr	cca Pro	576
				cct Pro												624
cag Gln	tct Ser 210	gcc Ala	ctg Leu	tct Ser	aaa Lys	gat Asp 215	ccc Pro	aac Asn	gaa Glu	aag Lys	aga Arg 220	gac Asp	cac His	atg Met	gtc Val	672

( )

ctg ctg gag ttt gtg acc gct gct ggg atc aca cat ggc atg gac gag
Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu
225 230 235 240

ctg tac aag tga
Leu Tyr Lys

<210> 70
<211> 243

<210> 70 <211> 243 <212> PRT <213> Artificial Sequence

<400> 70 Met Leu Leu Leu Leu Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 105 Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 150 Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro 185 Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr 200 Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val

Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 225 230 235 240

Leu Tyr Lys

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Ser	Val	Ser 35	Gly	Glu	Gly	Glu	Gly 40	Asp	Ala	Thr	Tyr	Gly 45	Lys	Leu	Thr	
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Asp	His	Met	Lys	Gln 85	His	Asp	Phe	Phe	Lys 90	Ser	Ala	Met	Pro	Glu 95	Gly	
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Thr	Arg	Ala 115	Glu	Val	Lys	Phe	Glu 120	Gly	Asp	Thr	Leu	Val 125	Asn	Arg	Ile	
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Lys	Gln	Lys	Asn	Gly 165	Ile	Lys	Val	Asn	Phe 170	Lys	Ile	Arg	His	Asn 175	Ile	
Glu	Asp	Gly	Ser 180	Val	Gln	Leu	Ala	Asp 185	His	Tyr	Gln	Gln	Asn 190	Thr	Pro	
Ile	Gly	Asp 195	Gly	Pro	Val	Leu	Leu 200	Pro	Asp	Asn	His	Tyr 205	Leu	Ser	Thr	
Gln	Ser 210	Ala	Leu	Ser	Lys	Asp 215	Pro	Asn	Glu	Lys	Arg 220	Asp	His	Met	Val	
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Ile	Gly	Asp 195	Gly	Pro	Val	Leu	Leu 200	Pro	Asp	Asn	His	Tyr 205	Leu	Ser	Thr	
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Val	Pro	Ile	Leu 20	Val	Glu	Leu	Asp	Gly 25	Asp	Val	Asn	Gly	His 30	Lys	Phe	
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Leu	Lys 50	Phe	Ile	Cys	Thr	Thr 55	Gly	Lys	Leu	Pro	Val 60	Pro	Trp	Pro	Thr	
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Lys	Gln	Lys	Asn	Gly 165	Ile	Lys	Va1	Asn	Phe 170	Lys	Ile	Arg	His	Asn 175	Ile	
Glu	Asp	Gly	Ser 180	Val	Gln	Leu	Ala	Asp 185	His	Tyr	Gln	Gln	Asn 190	Thr	Pro	
Ile	Gly	Asp 195	Gly	Pro	Val	Leu	Leu 200	Pro	Asp	Asn	His	Tyr 205	Leu	Ser	Thr	
Gln	Ser 210	Ala	Leu	Ser	Lys	Asp 215	Pro	Asn	Glu	Lys	Arg 220	Asp	His	Met	Val	
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Lys Leu Glu 145	Tyr Asn	Tyr Asn 150	Ser	His	Asn	Val 155	Tyr	Ile	Met	Ala	Asp 160	
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Ile Gly Asp 195	Gly Pro	Val Leu	Leu 200	Pro	Asp	Asn	His	Tyr 205	Leu	Ser	Thr	
Gln Ser Ala	Leu Ser	Lys Asp	Pro	Asn	Glu	Lys	Arg	Asp	His	Met	Val	

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Leu Tyr Lys

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tct gtc agc gga gag ggt gaa ggt gat gcc aca tac gga aag ctc acc 144 Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr

192 ctg aaa ttc atc tgc acc act gga aag ctc cct gtg cca tgg cca aca Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr

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432 gag ctg aag ggc att gac ttt aag gag gat gga aac att ctc ggc cac Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His 130

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aag caa aag aat ggc atc aag gtc aac ttc aag atc aga cac aac att 528 Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 165 170

gag gat gga tcc gtg cag ctg cag ctg gac cat tat caa cag aac act cca las follow Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro 190         576           atc ggc gac ggc ggc ctg tgt ctc ctc cca gac aac cat tac ggc gac ggc ggc ggc ggc ggc ctg gtg ctc ctc cca gac aac cat tac ctg tcc acc 195         624           cag tct gcc ctg tct aaa gat ccc aac gac aaa aag aga gac cac atg gtc gGln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val 210         672           ctg ctg gag ttt gtg acc gct gct ggg atc Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 225         720           ctg tac aag tga Leu Tyr Lys         732           <210> 78         2215 28           <2110> 78         2215 29           <2110> 243         2240           <2210> 78         2215 29           <2110> 243         2240           <2210> 78         2215 29           <2110 243         2240           <2210> 78         2215 29           <210> 78         2215 29           <2110 243         2220           <2210> 78         2215 29           <2110 243         2220           <2210> 78         2215 29           <2110 243         2220           <2210> 78         2215 29           <2110 243         2220           <2210 29         222           <210																	
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Cin Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val 210  Ctg ctg gag ttt gtg acc gct gct ggg atc acc act ggc atg gac gag Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 225  Ctg tac aag tga Leu Tyr Lys  C210> 78  <211> 243  <212> PRT  <213> Artificial Sequence  <400> 78  Met Phe Phe Phe Phe Phe Phe Ser Lys Gly Glu Glu Leu Phe Thr Gly Val 1  Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe 20  Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr 35  Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr 50  Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 65  Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly 85  Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 100  Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile		acc Thr	tcc Ser	ctg Leu	Tyr	cat His	aac Asn	gac Asp	cca Pro	Leu	ctc Leu	gtg Val	cct Pro	ggc	Asp	ggc Gly	atc Ile
Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 225  ctg tac aag tga Leu Tyr Lys  732  C210> 78  <211> 243  <212> PRT  <213> Artificial Sequence  <400> 78  Met Phe Phe Phe Phe Phe Phe Ser Lys Gly Glu Glu Leu Phe Thr Gly Val 15  Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe 20  Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr 35  Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr 50  Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 65  Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Gly Gly Val Gln Cys Phe Ser Arg Tyr Pro 80  Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 100  Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile	tc 672 al	gtc Val	atg Met	cac His	gac Asp	Arg	aag Lys	gaa Glu	aac Asn	ccc Pro	Asp	aaa Lys	tct Ser	ctg Leu	gcc Ala	Ser	cag Gln
C210> 78 C211> 243 C212> PRT C213> Artificial Sequence C400> 78 Met Phe Phe Phe Phe Phe Phe Ser Lys Gly Glu Glu Leu Phe Thr Gly Val 1	lú	Glu	gac Asp	atg Met	ggc Gly	cat His	Thr	atc Ile	ggg Gly	gct Ala	gct Ala	Thr	gtg Val	ttt Phe	gag Glu	ctg Leu	Leu
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	ys	Lys	Tyr		Gly	Asp	Asp	Lys		Phe	Ile	Thr	Arg		Gln	Val	Tyr
	le	Ile	Arg	Asn		Leu	Thr	Asp	Gly		Phe	Lys	Val	Glu		Arg	Thr
Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His 130 135 140	is	His	Gly	Leu	Ile		Gly	Asp	G1u	Lys		Asp	Ile	Gly	Lys		Glu
Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 145 150 155 160			Ala	Met	Ile	Tyr		Asn	His	Ser	Asn		Asn	Tyr	Glu	Leu	
Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 165 170 175	le	Ile		His	Arg	Ile	Lys		Asn	Val	Lys	Ile		Asn	Lys	Gln	Lys

Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro  $180 \,$ 

Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr

205 195 200 Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val 220 Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 230 225 Leu Tyr Lys <210> 79 <211> 732 <212> DNA <213> Artificial Sequence <223> Description of Artificial Sequence: Pro(CCC)5GFP <220> <221> CDS <222> (1)..(732) 48 atg ccc ccc ccc ccc agc aag ggc gag gaa ctg ttc act ggc gtg Met Pro Pro Pro Pro Pro Ser Lys Gly Glu Leu Phe Thr Gly Val gtc cca att ctc gtg gaa ctg gat ggc gat gtg aat ggg cac aaa ttt 96 Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe 20 tct gtc agc gga gag ggt gaa ggt gat gcc aca tac gga aag ctc acc 144 Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr 35 192 ctg aaa ttc atc tgc acc act gga aag ctc cct gtg cca tgg cca aca Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr 50 ctg qtc act acc ttc tct tat ggc gtg cag tgc ttt tcc aga tac cca 240 Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 288 gac cat atg aag cag cat gac ttt ttc aag agc gcc atg ccc gag ggc Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly tat gtg cag gag aga acc atc ttt ttc aaa gat gac ggg aac tac aag 336 Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 384 acc cgc gct gaa gtc aag ttc gaa ggt gac acc ctg gtg aat aga atc Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile 120 432 gag ctg aag ggc att gac ttt aag gag gat gga aac att ctc ggc cac Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His 135 aaq ctq qaa tac aac tat aac tcc cac aat qtq tac atc atg gcc gac 480 Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp

150

aag Lys	caa Gln	aag Lys	aat Asn	ggc Gly 165	atc Ile	aag Lys	gtc Val	aac Asn	ttc Phe 170	aag Lys	atc Ile	aga Arg	cac His	aac Asn 175	att Ile	528
gag Glu	gat Asp	gga Gly	tcc Ser 180	gtg Val	cag Gln	ctg Leu	gcc Ala	gac Asp 185	cat His	tat Tyr	caa Gln	cag Gln	aac Asn 190	act Thr	cca Pro	576
atc Ile	ggc Gly	gac Asp 195	ggc Gly	cct Pro	gtg Val	ctc Leu	ctc Leu 200	cca Pro	gac Asp	aac Asn	cat His	tac Tyr 205	ctg Leu	tcc Ser	acc Thr	624
cag Gln	tct Ser 210	gcc Ala	ctg Leu	tct Ser	aaa Lys	gat Asp 215	ccc Pro	aac Asn	gaa Glu	aag Lys	aga Arg 220	gac Asp	cac His	atg Met	gtc Val	672
ctg Leu 225	ctg Leu	gag Glu	ttt Phe	gtg Val	acc Thr 230	gct Ala	gct Ala	.GJÀ āāā	atc Ile	aca Thr 235	cat His	ggc Gly	atg Met	gac Asp	gag Glu 240	720
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Val	Pro	Ile	Leu 20	Val	Glu	Leu	Asp	Gly 25	Asp	Val	Asn	Gly	His 30	Lys	Phe	
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Leu	Lys 50	Phe	Ile	Cys	Thr	Thr 55	Gly	Lys	Leu	Pro	Val 60	Pro	Trp	Pro	Thr	
Leu 65	Val	Thr	Thr	Phe	Ser 70	Tyr	Gly	Val	Gln	Cys 75	Phe	Ser	Arg	Tyr	Pro 80	
Asp	His	Met	Lys	Gln 85	His	Asp	Phe	Phe	Lys 90	Ser	Ala	Met	Pro	Glu 95	Gly	
Tyr	Val	Gln	Glu 100	Arg	Thr	Ile	Phe	Phe 105	Lys	Asp	Asp	Gly	Asn 110	Tyr	Lys	
Thr	Arg	Ala 115	Glu	Val	Lys	Phe	Glu 120	Gly	Asp	Thr	Leu	Val 125	Asn	Arg	Ile	
Glu	Leu 130	Lys	Gly	Ile	Asp	Phe 135	Lys	Glu	Asp	Gly	Asn 140	Ile	Leu	Gly	His	
Lys 145	Leu	Glu	Tyr	Asn	Tyr 150	Asn	Ser	His	Asn	Val 155	Tyr	Ile	Met	Ala	Asp 160	
Lys	Gln	Lys	Asn	Gly 165	Ile	Lys	Val	Asn	Phe 170	Lys	Ile	Arg	His	Asn 175	Ile	
Glu	Asp	Gly	Ser	Val	Gln	Leu	Ala	Asp	His	Tyr	Gln	Gln	Asn	Thr	Pro	

180 185 190

Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr 195 200 205

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tct gtc agc gga gag ggt gaa ggt gat gcc aca tac gga aag ctc acc 144 Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr

ctg aaa ttc atc tgc acc act gga aag ctc cct gtg cca tgg cca aca 192 Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr
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ctg gtc act acc ttc tct tat ggc gtg cag tgc ttt tcc aga tac cca 240 Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 65 70 75 80

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acc cgc gct gaa gtc aag ttc gaa ggt gac acc ctg gtg aat aga atc 384
Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile
115 120 125

gag ctg aag ggc att gac ttt aag gag gat gga aac att ctc ggc cac 432 Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His 130 135

aag ctg gaa tac aac tat aac tcc cac aat gtg tac atc atg gcc gac 480

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atc Ile	ggc Gly	gac Asp 195	ggc Gly	cct Pro	gtg Val	ctc Leu	ctc Leu 200	cca Pro	gac Asp	aac Asn	cat His	tac Tyr 205	ctg Leu	tcc Ser	acc Thr	624
cag Gln	tct Ser 210	gcc Ala	ctg Leu	tct Ser	aaa Lys	gat Asp 215	ccc Pro	aac Asn	gaa Glu	aag Lys	aga Arg 220	gac Asp	cac His	atg Met	gtc Val	672
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Met 1 Val Ser Leu 65 Asp	Pro Pro Val Lys 50 Val His	Pro Ile Ser 35 Phe Thr Met	Leu 20 Gly Ile Thr Lys Glu 100	5 Val Glu Cys Phe Gln 85 Arg	Glu Gly Thr Ser 70 His	Leu Glu Thr 55 Tyr Asp Ile	Asp Gly 40 Gly Gly Phe	Gly 25 Asp Lys Val Phe	Asp Ala Leu Gln Lys 90 Lys	Val Thr Pro Cys 75 Ser Asp	Asn Tyr. Val 60 Phe	Gly Gly 45 Pro Ser Met Gly	His 30 Lys Trp Arg Pro	Leu Pro Tyr Glu 95 Tyr	Phe Thr Thr Pro 80 Gly Lys	
Met 1 Val Ser Leu 65 Asp Tyr	Pro Pro Val Lys 50 Val His Val Arg	Pro Ile Ser 35 Phe Thr Met Gln Ala 115	Leu 20 Gly Ile Thr Lys Glu 100 Glu	5 Val Glu Cys Phe Gln 85 Arg Val	Glu Gly Thr Ser 70 His Thr	Leu Glu Thr 55 Tyr Asp Ile . Phe	Asp Gly 40 Gly Gly Phe Phe Glu 120	Gly 25 Asp Lys Val Phe 105 Gly	Asp Ala Leu Gln Lys 90 Lys Asp	Val Thr Pro Cys 75 Ser Asp	Asn Tyr. Val 60 Phe Ala Asp	Gly Gly 45 Pro Ser Met Gly Val 125	His 30 Lys Trp Arg Pro Asn 110	Lys Leu Pro Tyr Glu 95 Tyr Arg	Phe Thr Thr Pro 80 Gly Lys Ile	
Met 1 Val Ser Leu 65 Asp Tyr Thr	Pro Pro Val Lys 50 Val His Val Arg Leu 130	Pro Ile Ser 35 Phe Thr Met Gln Ala 115 Lys	Leu 20 Gly Ile Thr Lys Glu 100 Glu Gly	5 Val Glu Cys Phe Gln 85 Arg Val Ile	Glu Gly Thr Ser 70 His Thr Lys	Leu Glu Thr 55 Tyr Asp Ile Phe 135	Asp Gly 40 Gly Gly Phe Phe Lys	Gly 25 Asp Lys Val Phe 105 Gly	Asp Ala Leu Gln Lys 90 Lys Asp	Val Thr Pro Cys 75 Ser Asp Thr	Asn Tyr. Val 60 Phe Ala Asp Leu Asn	Gly Gly 45 Pro Ser Met Gly Val 125 Ile	His 30 Lys Trp Arg Pro Asn 110	Lys Leu Pro Tyr Glu 95 Tyr Arg	Phe Thr Thr Pro 80 Gly Lys Ile His	

165 170 Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro 185 Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys <210> 83 <211> 732 <212> DNA <213> Artificial Sequence <223> Description of Artificial Sequence: Pro(CCT)5GFP <221> CDS <222> (1)..(732) <400> 83 48 atg cct cct cct cct agc aag ggc gag gaa ctg ttc act ggc gtg Met Pro Pro Pro Pro Pro Ser Lys Gly Glu Glu Leu Phe Thr Gly Val gtc cca att ctc gtg gaa ctg gat ggc gat gtg aat ggg cac aaa ttt Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe 96 144 tct qtc agc qga gag ggt gaa ggt gat gcc aca tac gga aag ctc acc Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr ctg aaa ttc atc tgc acc act gga aag ctc cct gtg cca tgg cca aca 192 Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr ctg gtc act acc ttc tct tat ggc gtg cag tgc ttt tcc aga tac cca 240 Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro gac cat atg aag cag cat gac ttt ttc aag agc gcc atg ccc gag ggc Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly 288 85 90 tat gtg cag gag aga acc atc ttt ttc aaa gat gac ggg aac tac aag 336 Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 105 384 acc ege get gaa gte aag tte gaa ggt gae ace etg gtg aat aga ate

Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile 120

gag ctg aag ggc att gac ttt aag gag gat gga aac att ctc ggc cac Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His

125

432

130			135					140					
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atc ggc gac Ile Gly Asp 195	ggc cct Gly Pro	gtg Val	ctc Leu	ctc Leu 200	cca Pro	gac Asp	aac Asn	cat His	tac Tyr 205	ctg Leu	tcc Ser	acc Thr	624
cag tct gcc Gln Ser Ala 210	ctg tct Leu Ser	aaa Lys	gat Asp 215	ccc Pro	aac Asn	gaa Glu	aag Lys	aga Arg 220	gac Asp	cac His	atg Met	gtc Val	672
ctg ctg gag Leu Leu Glu 225	ttt gto Phe Val	acc Thr 230	gct Ala	gct Ala	ggg Gly	atc Ile	aca Thr 235	cat His	ggc Gly	atg Met	gac Asp	gag Glu 240	720
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160 150 155 145 Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro 185 Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys <210> 85 <211> 732 <212> DNA <213> Artificial Sequence <223> Description of Artificial Sequence: Pro(CGA)5GFP <220> <221> CDS <222> (1)..(732) atg cga cga cga cga agc aag ggc gag gaa ctg ttc act ggc gtg 48 Met Arg Arg Arg Arg Ser Lys Gly Glu Glu Leu Phe Thr Gly Val 96 gtc cca att ctc gtg gaa ctg gat ggc gat gtg aat ggg cac aaa ttt Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe tet gte age gga gag ggt gaa ggt gat gee aca tae gga aag ete ace 144 Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr 192 ctg aaa ttc atc tgc acc act gga aag ctc cct gtg cca tgg cca aca Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr 240 ctg gtc act acc ttc tct tat ggc gtg cag tgc ttt tcc aga tac cca Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro gac cat atg aag cag cat gac ttt ttc aag agc gcc atg ccc gag ggc 288 Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly 336 tat gtg cag gag aga acc atc ttt ttc aaa gat gac ggg aac tac aag Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 100 105 acc cgc gct gaa gtc aag ttc gaa ggt gac acc ctg gtg aat aga atc 384 Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile 115 120 125

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						- 1	Dh.a	D1	T	7.00	70	Clu	7 cn	Ф	T	
Tyr	Val	Gln	Glu 100	Arg	Thr	TIE	Pne	105	гàг	ASP	Asp	СТУ	110	ıyı	гуѕ	
			100					105					110	Arg		

135 140 130 Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 150 155 Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 170 Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val 215 Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys <210> 87 <211> 732 <212> DNA <213> Artificial Sequence <220> <223> Description of Artificial Sequence: Ser(AGC)5GFP <221> CDS <222> (1) .. (732) <400> 87 atg agc agc agc agc agc aag ggc gag gaa ctg ttc act ggc gtg 48 Met Ser Ser Ser Ser Ser Lys Gly Glu Glu Leu Phe Thr Gly Val gtc cca att ctc gtg gaa ctg gat ggc gat gtg aat ggg cac aaa ttt 96 Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe tct gtc agc gga gag ggt gaa ggt gat gcc aca tac gga aag ctc acc 144 Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr - 40 192 ctg aaa ttc atc tgc acc act gga aag ctc cct gtg cca tgg cca aca Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr 55 60 240 ctg gtc act acc ttc tct tat ggc gtg cag tgc ttt tcc aga tac cca Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro gac cat atg aag cag cat gac ttt ttc aag agc gcc atg ccc gag ggc 288 Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly 336 tat gtg cag gag aga acc atc ttt ttc aaa gat gac ggg aac tac aag Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys

	_	_	_	_	_		gaa Glu 120		-		_					384
							aag Lys									432
							tcc Ser									480
							gtc Val									528
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Ser	Val	Ser 35	Gly	Glu	Gly	Glu	Gly 40	Asp	Ala	Thr	Tyr	Gly 45	Lys	Leu	Thr	
Leu	Lys 50	Phe	Ile	Cys	Thr	Thr 55	Gly	Lys	Leu	Pro	Val 60	Pro	Trp	Pro	Thr	
Leu 65	Val	Thr	Thr	Phe	Ser 70	Tyr	Gly	Val	Gln	Cys 75	Phe	Ser	Arg	Tyr	Pro 80	
7																
Asp	His	Met	Lys	Gln 85	His	Asp	Phe	Phe	Lys 90	Ser	Ala	Met	Pro	Glu 95	Gly	

Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile

120 125 115 Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His 135 Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 150 155 Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr 200 195 Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 230 Leu Tyr Lys <210> 89 <211> 732 <212> DNA <213> Artificial Sequence <220> <223> Description of Artificial Sequence: Ser(AGT)5GFP <220> <221> CDS <222> (1)..(732) <400> 89 48 atg agt agt agt agt agc aag ggc gag gaa ctg ttc act ggc gtg Met Ser Ser Ser Ser Ser Lys Gly Glu Leu Phe Thr Gly Val 96 gtc cca att ctc gtg gaa ctg gat ggc gat gtg aat ggg cac aaa ttt Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe 20 144 tct gtc agc gga gag ggt gaa ggt gat gcc aca tac gga aag ctc acc Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr 192 ctg aaa ttc atc tgc acc act gga aag ctc cct gtg cca tgg cca aca Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr ctg gtc act acc ttc tct tat ggc gtg cag tgc ttt tcc aga tac cca 240 Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 288 gac cat atg aag cag cat gac ttt ttc aag agc gcc atg ccc gag ggc Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly tat gtg cag gag aga acc atc ttt ttc aaa gat gac ggg aac tac aag 336

Tyr	Val	Gln	Glu 100	Arg	Thr	Ile	Phe	Phe 105	Lys	Asp	Asp	Gly	Asn 110	Tyr	Lys	
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gag Glu	ctg Leu 130	aag Lys	ggc Gly	att Ile	gac Asp	ttt Phe 135	aag Lys	gag Glu	gat Asp	gga Gly	aac Asn 140	att Ile	ctc Leu	ggc Gly	cac His	432
aag Lys 145	ctg Leu	gaa Glu	tac Tyr	aac Asn	tat Tyr 150	aac Asn	tcc Ser	cac His	aat Asn	gtg Val 155	tac Tyr	atc Ile	atg Met	gcc Ala	gac Asp 160	480
aag Lys	caa Gln	aag Lys	aat Asn	ggc Gly 165	atc Ile	aag Lys	gtc Val	aac Asn	ttc Phe 170	aag Lys	atc Ile	aga Arg	cac His	aac Asn 175	att Ile	528
gag Glu	gat Asp	gga Gly	tcc Ser 180	gtg Va:l	cag Gln	ctg Leu	gcc Ala	gac Asp 185	cat His	tat Tyr	caa Gln	cag Gln	aac Asn 190	act Thr	cca Pro	576
atc Ile	ggc Gly	gac Asp 195	ggc Gly	cct Pro	gtg Val	ctc Leu	ctc Leu 200	cca Pro	gac Asp	aac Asn	cat His	tac Tyr 205	ctg Leu	tcc Ser	acc Thr	624
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Ser	Val	Ser 35	Gly	Glu	Gly	Glu	Gly 40	Asp	Ala	Thr	Tyr	Gly 45	Lys	Leu	Thr	
Leu	Lys 50	Phe	Ile	Cys	Thr	Thr 55	Gly	Lys	Leu	Pro	Val 60	Pro	Trp	Pro	Thr	
Leu 65	Val	Thr	Thr	Phe	Ser 70	Tyr	Gly	Val	Gln	Cys 75	Phe	Ser	Arg	Tyr	Pro 80	
Asp	His	Met	Lys	Gln 85	His	Asp	Phe	Phe	Lys 90	Ser	Ala	Met	Pro	Glu 95	Gly	
_				_					_	_	_		_			

Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys

- xciii -100 105 110 Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile 120 Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 230 Leu Tyr Lys <210> 91 <211> 732 <212> DNA <213> Artificial Sequence <223> Description of Artificial Sequence: Ser(TCA)5GFP <220> <221> CDS <222> (1)..(732) <400> 91 48 atg tca tca tca tca agc aag ggc gag gaa ctg ttc act ggc gtg Met Ser Ser Ser Ser Ser Lys Gly Glu Glu Leu Phe Thr Gly Val gtc cca att ctc gtg gaa ctg gat ggc gat gtg aat ggg cac aaa ttt Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe tct gtc agc gga gag ggt gaa ggt gat gcc aca tac gga aag ctc acc Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr

ctg aaa ttc atc tgc acc act gga aag ctc cct gtg cca tgg cca aca Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr ctg gtc act acc ttc tct tat ggc gtg cag tgc ttt tcc aga tac cca Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro gac cat atg aag cag cat gac ttt ttc aag agc gcc atg ccc gag ggc Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly - xciv -

8	5	90	95												
tat gtg cag gag ag Tyr Val Gln Glu Ar 100	a acc atc tt g Thr Ile Ph	t ttc aaa gat e Phe Lys Asp 105	gac ggg aac tac aag 336 Asp Gly Asn Tyr Lys 110												
acc cgc gct gaa gt Thr Arg Ala Glu Va 115	c aag ttc ga l Lys Phe Gl 12	u Gly Asp Thr	ctg gtg aat aga atc 384 Leu Val Asn Arg Ile 125												
gag ctg aag ggc at Glu Leu Lys Gly Il 130	t gac ttt aa e Asp Phe Ly 135	ng gag gat gga vs Glu Asp Gly	aac att ctc ggc cac 432 Asn Ile Leu Gly His 140												
aag ctg gaa tac aa Lys Leu Glu Tyr As 145	c tat aac tc n Tyr Asn Se 150	cc cac aat gtg er His Asn Val 155	tac atc atg gcc gac 480 Tyr Ile Met Ala Asp 160												
aag caa aag aat gg Lys Gln Lys Asn Gl 16	y Ile Lys Va	c aac ttc aag l Asn Phe Lys 170	atc aga cac aac att 528 Ile Arg His Asn Ile 175												
gag gat gga tcc gt Glu Asp Gly Ser Va 180	g cag ctg gc l Gln Leu Al	cc gac cat tat a Asp His Tyr 185	caa cag aac act cca 576 Gln Gln Asn Thr Pro 190												
atc ggc gac ggc cc Ile Gly Asp Gly Pr 195	t gtg ctc ct o Val Leu Le 20	u Pro Asp Asn	cat tac ctg tcc acc 624 His Tyr Leu Ser Thr 205												
cag tct gcc ctg tc Gln Ser Ala Leu Se 210	t aaa gat cc r Lys Asp Pr 215	c aac gaa aag o Asn Glu Lys	aga gac cac atg gtc 672 Arg Asp His Met Val 220												
ctg ctg gag ttt gt Leu Leu Glu Phe Va 225	g acc gct gc l Thr Ala Al 230	et ggg atc aca a Gly Ile Thr 235	cat ggc atg gac gag 720 His Gly Met Asp Glu 240												
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Ser Val Ser Gly Gl 35		y Asp Ala Thr	Tyr Gly Lys Leu Thr 45												
Leu Lys Phe Ile Cy 50	s Thr Thr Gl 55	y Lys Leu Pro	Val Pro Trp Pro Thr 60												
Leu Val Thr Thr Ph 65	e Ser Tyr Gl 70	y Val Gln Cys 75	Phe Ser Arg Tyr Pro 80												
Asp His Met Lys Gl	n His Asp Ph	ne Phe Lys Ser	Ala Met Pro Glu Gly												

85 90 95

Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 100 105 110

Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile 115 120 125

Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His 130 135 140

Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 145 150 155 160

Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 165 170 175

Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro 180 185 190

Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr 195 200 205

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tct gtc agc gga gag ggt gaa ggt gat gcc aca tac gga aag ctc acc 144 Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr 35 40 45

ctg aaa ttc atc tgc acc act gga aag ctc cct gtg cca tgg cca aca 192 Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr 50 55 60

ctg gtc act acc ttc tct tat ggc gtg cag tgc ttt tcc aga tac cca 240 Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 65 70 75 80

gac cat a Asp His M	tg aag et Lys	cag Gln 85	cat His	gac Asp	ttt Phe	ttc Phe	aag Lys 90	agc Ser	gcc Ala	atg Met	ccc Pro	gag Glu 95	ggc Gly	288
tat gtg c Tyr Val G														336
acc cgc g Thr Arg A 1	ct gaa la Glu 15	gtc Val	aag Lys	ttc Phe	gaa Glu 120	ggt Gly	gac Asp	acc Thr	ctg Leu	gtg Val 125	aat Asn	aga Arg	atc Ile	384
gag ctg a Glu Leu L 130														432
aag ctg g Lys Leu G 145	aa tac lu Tyr	aac Asn	tat Tyr 150	aac Asn	tcc Ser	cac His	aat Asn	gtg Val 155	tac Tyr	atc Ile	atg Met	gcc Ala	gac Asp 160	480
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gag gat g Glu Asp G	ga tcc ly Ser 180	gtg Val	cag Gln	ctg Leu	gcc Ala	gac Asp 185	cat His	tat Tyr	caa Gln	cag Gln	aac Asn 190	act Thr	cca Pro	576
atc ggc g Ile Gly A 1														624
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ctg tac a Leu Tyr L														732
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Ser Val Se	er Gly 35	Glu	Gly	Glu	Gly 40	Asp	Ala	Thr	Tyr	Gly 45	Lys	Leu	Thr	
Leu Lys Pl 50	he Ile	Cys	Thr	Thr 55	Gly	Lys	Leu	Pro	Val 60	Pro	Trp	Pro	Thr	
Leu Val T	hr Thr	Phe	Ser	Tyr	Gly	Val	Gln	Cys	Phe	Ser	Arg	Tyr	Pro	

70 80 65 75 Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile 115 120 Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 170 Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr 195 200 Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 230 Leu Tyr Lys <210> 95 <211> 732 <212> DNA <213> Artificial Sequence <223> Description of Artificial Sequence: Ser(TCG)5GFP <220> <221> CDS <222> (1)..(732) atg tcg tcg tcg tcg agc aag ggc gag gaa ctg ttc act ggc gtg 48 Met Ser Ser Ser Ser Ser Lys Gly Glu Glu Leu Phe Thr Gly Val gtc cca att ctc gtg gaa ctg gat ggc gat gtg aat ggg cac aaa ttt 96 Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe 20 30 tct gtc agc gga gag ggt gaa ggt gat gcc aca tac gga aag ctc acc 144 Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr ctg aaa ttc atc tgc acc act gga aag ctc cct gtg cca tgg cca aca 192 Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr 50

	gtc Val															240
	cat His															288
	gtg Val															336
	cgc Arg															384
	ctg Leu 130															432
	ctg Leu															480
	caa Gln															528
	gat Asp															576
	ggc Gly															624
	tct Ser 210															672
	ctg Leu															720
ctg Leu	tac Tyr	aag Lys	tga													732
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Val	Pro	Ile	Leu 20	Val	Glu	Leu	Asp	Gly 25	Asp	Val	Asn	Gly	His 30	Lys	Phe	
Ser	Val	Ser 35	Gly	Glu	Gly	Glu	Gly 40	Asp	Ala	Thr	Tyr	Gly 45	Lys	Leu	Thr	

Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr

50 55 60 Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 105 110 Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 155 Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro 180 185 Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys <210> 97 <211> 732 <212> DNA <213> Artificial Sequence <223> Description of Artificial Sequence: Ser(TCT)5GFP <220> <221> CDS <222> (1)..(732) <400> 97 atg tet tet tet tet age aag gge gag gaa etg tte aet gge gtg 48 Met Ser Ser Ser Ser Ser Lys Gly Glu Glu Leu Phe Thr Gly Val 96 gtc cca att ctc gtg gaa ctg gat ggc gat gtg aat ggg cac aaa ttt Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe 25

tct gtc agc gga gag ggt gaa ggt gat gcc aca tac gga aag ctc acc

Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr

ctg aaa ttc atc tgc acc act gga aag ctc cct gtg cca tgg cca aca

144

192

Leu	Lys 50	Phe	Ile	Cys ·	Thr	Thr 55	Gly	Lys	Leu	Pro	Val 60	Pro	Trp	Pro	Thr	
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							ttt Phe									288
tat Tyr	gtg Val	cag Gln	gag Glu 100	aga Arg	acc Thr	atc Ile	ttt Phe	ttc Phe 105	aaa Lys	gat Asp	gac Asp	Gly ggg	aac Asn 110	tac Tyr	aag Lys	336
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gag Glu	ctg Leu 130	aag Lys	ggc Gly	att Ile	gac Asp	ttt Phe 135	aag Lys	gag Glu	gat Asp	gga Gly	aac Asn 140	att Ile	ctc Leu	ggc Gly	cac His	432
aag Lys 145	ctg Leu	gaa Glu	tac Tyr	aac Asn	tat Tyr 150	aac Asn	tcc Ser	cac His	aat Asn	gtg Val 155	tac Tyr	atc Ile	atg Met	gcc Ala	gac Asp 160	480
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gag Glu	gat Asp	gga Gly	tcc Ser 180	gtg Val	cag Gln	ctg Leu	gcc Ala	gac Asp 185	cat His	tat Tyr	caa Gln	cag Gln	aac Asn 190	act Thr	cca Pro	576
atc Ile	ggc Gly	gac Asp 195	ggc Gly	cct Pro	gtg Val	ctc Leu	ctc Leu 200	cca Pro	gac Asp	aac Asn	cat His	tac Tyr 205	ctg Leu	tcc Ser	acc Thr	624
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							gct Ala									720
-	tac Tyr	aag Lys	tga													732
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1	JUL	JUL	JUL	5	JU1	501	בעם	<b>-</b> 1	10	Jiu	LC u	2110		15		

10

Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe 20 25 30

Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr

45 35 Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His 135 Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 155 Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 165 Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro 185 Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr 200 205 Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 230 235 Leu Tyr Lys <210> 99 <211> 732 <212> DNA <213> Artificial Sequence <223> Description of Artificial Sequence: Thr(ACA)5GFP <220> <221> CDS <222> (1)..(732) <400> 99 atg aca aca aca aca agc aag ggc gag gaa ctg ttc act ggc gtg Met Thr Thr Thr Thr Ser Lys Gly Glu Glu Leu Phe Thr Gly Val 5 96 gtc cca att ctc gtg gaa ctg gat ggc gat gtg aat ggg cac aaa ttt Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe tct gtc agc gga gag ggt gaa ggt gat gcc aca tac gga aag ctc acc Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr

35		40	)		45	
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gac cat atg Asp His Met						
tat gtg cag Tyr Val Gln						-
acc cgc gct Thr Arg Ala 115			ı Gly Asp	Thr Leu V		
gag ctg aag Glu Leu Lys 130						
aag ctg gaa Lys Leu Glu 145	Tyr Asn T					
aag caa aag Lys Gln Lys						
gag gat gga Glu Asp Gly			-		-	
atc ggc gac Ile Gly Asp 195		-	Pro Asp	Asn His T	_	
cag tct gcc Gln Ser Ala 210	-	-	_			-
ctg ctg gag Leu Leu Glu 225	Phe Val Th					
ctg tac aag Leu Tyr Lys	tga					732
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20 25 30

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Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr 50 55 60

Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 65 70 75 80

Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly 85 90 95

Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 100 105 110

Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile 115 120 125

Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His 130 135 140

Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 145 150 155 160

Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 165 170 175

Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro 180 185 190

Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr 195 200 205

Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val 210 215 220

Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 225 230 235 240

Leu Tyr Lys

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<223> Description of Artificial Sequence: Thr(ACC)5GFP

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<221> CDS

<222> (1)..(732)

<400> 101

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ctg aaa ttc atc Leu Lys Phe Ile 50	Cys Int I	ct gga aa nr Gly Ly 55	g ctc cct s Leu Pro	gtg cca tgg ( Val Pro Trp ) 60	cca aca 192 Pro Thr
ctg gtc act acc Leu Val Thr Thr	ttc tct ta Phe Ser Ty 70	at ggc gt yr Gly Va	g cag tgc l Gln Cys 75	ttt tcc aga Phe Ser Arg	tac cca 240 Tyr Pro 80
65 gac cat atg aag Asp His Met Lys	cag cat g Gln His A	ac ttt tt sp Phe Ph	c aag agc ne Lys Ser 90	gcc atg ccc Ala Met Pro	gag ggc 288 Glu Gly 95
tat gtg cag gag Tyr Val Gln Glu 100	aga acc a Arg Thr I	TC 1110 11	c aaa gat ne Lys Asp 05	gac ggg aac Asp Gly Asn 110	tac aag 336 Tyr Lys
acc cgc gct gaa Thr Arg Ala Glu 115		tc gaa go he Glu G 120	gt gac acc ly Asp Thr	ctg gtg aat Leu Val Asn 125	aga atc 384 Arg Ile
gag ctg aag ggc Glu Leu Lys Gly	ITE Wab	tt aag g Phe Lys G 135	ag gat gga lu Asp Gly	a aac att ctc y Asn Ile Leu 140	ggc cac 432 Gly His
130 aag ctg gaa tac Lys Leu Glu Ty			ac aat gto is Asn Va 15	g tac atc atg l Tyr Ile Met 5	gcc gac 480 Ala Asp 160
145 aag caa aag aa Lys Gln Lys As:		aag gtc a Lys Val <i>F</i>	ac ttc aa Asn Phe Ly 170	g atc aga cac s Ile Arg His	aac att 528 Asn Ile 175
gag gat gga tc Glu Asp Gly Se 18	c gtg cag r Val Gln	Den Pro	gac cat ta Asp His Ty 185	t caa cag aac r Gln Gln Asr 190	act cca 576 Thr Pro
atc ggc gac gg Ile Gly Asp Gl		ctc ctc c Leu Leu 200	cca gac aa Pro Asp As	ac cat tac cto on His Tyr Leo 205	tcc acc 624 Ser Thr
cag tct gcc ct Gln Ser Ala Le	g tot aaa eu Ser Lys	gat ccc Asp Pro 215	aac gaa aa Asn Glu Ly	ag aga gac ca ys Arg Asp Hi 220	e atg gtc 672 s Met Val
ctg ctg gag tt Leu Leu Glu Pl	tt gtg acc ne Val Thr 230	ATO ME	ggg atc a Gly Ile T	ca cat ggc at hr His Gly Me 35	g gac gag 720 t Asp Glu 240
225 ctg tac aag t Leu Tyr Lys	ga			·	73

<210> 102

<211> 243 <212> PRT

<213> Artificial Sequence

Met Thr Thr Thr Thr Ser Lys Gly Glu Glu Leu Phe Thr Gly Val

- cv -10 Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 70 Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 100 Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile 120 Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 165 Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro 185 Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 230 225 Leu Tyr Lys <210> 103 <211> 732 <212> DNA <213> Artificial Sequence <223> Description of Artificial Sequence: Thr(ACG)5GFP <220>

<221> CDS
<222> (1)..(732)

<400> 103
atg acg acg acg acg agc aag ggc gag gaa ctg ttc act ggc gtg

48
Met Thr Thr Thr Thr Ser Lys Gly Glu Glu Leu Phe Thr Gly Val

1 5

gtc Val	cca Pro	att Ile	ctc Leu 20	gtg Val	gaa Glu	ctg Leu	gat Asp	ggc Gly 25	gat Asp	gtg Val	aat Asn	ggg Gly	cac His 30	aaa Lys	ttt Phe	96
tct Ser	gtc Val	agc Ser 35	gga Gly	gag Glu	ggt Gly	gaa Glu	ggt Gly 40	gat Asp	gcc Ala	aca Thr	tac Tyr	gga Gly 45	aag Lys	ctc Leu	acc Thr	144
ctg Leu	aaa Lys 50	ttc Phe	atc Ile	tgc Cys	acc Thr	act Thr 55	gga Gly	aag Lys	ctc Leu	cct Pro	gtg Val 60	cca Pro	tgg Trp	cca Pro	aca Thr	192
ctg Leu 65	gtc Val	act Thr	acc Thr	ttc Phe	tct Ser 70	tat Tyr	ggc Gly	gtg Val	cag Gln	tgc Cys 75	ttt Phe	tcc Ser	aga Arg	tac Tyr	cca Pro 80	240
gac Asp	cat His	atg Met	aag Lys	cag Gln 85	cat His	gac Asp	ttt Phe	ttc Phe	aag Lys 90	agc Ser	gcc Ala	atg Met	ccc Pro	gag Glu 95	ggc Gly	288
tat Tyr	gtg Val	cag Gln	gag Glu 100	Arg	acc Thr	atc Ile	ttt Phe	ttc Phe 105	aaa Lys	gat Asp	gac Asp	ggg Gly	aac Asn 110	tac Tyr	aag Lys	336
Thr	Arg	Ala 115	Glu	Val	гÀг	Pne	120	GIY	rsp	1111	Бец	gtg Val 125				384
Glu	Leu 130	Lys	Gly	lle	Asp	135	ьуѕ	GIU	АЗР	Gry	140					432
Lys 145	Leu	Glu	туг	: Asn	150	Asn	Ser	HIS	ASII	155	. 191	atc Ile		• •	160	480
Lys	Gln	Lys	. Asr	165	lle	. Lys	val	ASI	170	)	, 110	aga Arg		175		528
Glu	Asp	Gly	/ Sei	c Val	. Gln	Lev	ı A±a	185	) пте	, 1 <b>y</b> 1	. 611	, OII	190	)	cca Pro	576
Ile	e Gly	/ Asp 199	o Gly	y Pro	val	ьer	200	)	, ASE	, Kai	1 1111	205	,		acc Thr	624
caq Glr	g tot n Sei 210	r Ala	c cte a Le	g toi u Se:	t aaa r Lys	a gat s Asp 21	o Pro	c aad o Asr	gaa Glu	a aaq ı Ly:	g aga s Arc 22	a war	c cac	c ato s Met	g gtc : Val	672
cto Lev 22	ي Le	g ga u Gl	g tt u Ph	t gte e Va	g acc 1 Th: 230	r Ala	t gci a Ala	t ggo a Gl	g ate y Ile	c aca e Thi 23	L UT	t ggo s Gly	c ate	g gad t Asp	gag Glu 240	
-	g ta u Ty		-	a												732

<210> 104 <211> 243

<212> PRT <213> Artificial Sequence

Met Thr Thr Thr Thr Ser Lys Gly Glu Glu Leu Phe Thr Gly Val

Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe

Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr

Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr

Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 70

Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly

Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 1.05

Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile

Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His 135

Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 155 150 145

Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 170

Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro

Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr

Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val 215

Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 235

Leu Tyr Lys

<210> 105

<211> 732

<212> DNA <213> Artificial Sequence

<223> Description of Artificial Sequence: Thr(ACT)5GFP

<220>

<221> CDS

<222> (1)..(732)

atg act act act act agc aag ggc gag gaa ctg ttc act ggc gtg <400> 105 48

,,,		- 04111			
Met Thr Thr Thr	Thr Thr Ser	Lys Gly Glu	ı Glu Leu Phe	Thr Gly Val 15	
gtc cca att ctc Val Pro Ile Leu 20	gtg gaa ctg Val Glu Leu	gat ggc gat Asp Gly Asp 25	t gtg aat ggg o Val Asn Gly	cac aaa ttt His Lys Phe 30	96
tct gtc agc gga Ser Val Ser Gly	gag ggt gaa Glu Gly Glu	ggt gat gc Gly Asp Al	c aca tac gga a Thr Tyr Gly 45	aag ctc acc Lys Leu Thr	144
ctg aaa ttc atc Leu Lys Phe Ile	tgc acc act Cys Thr Thr	017 -7-	c cct gtg cca u Pro Val Pro 60	tgg cca aca Trp Pro Thr	192
50 ctg gtc act acc Leu Val Thr Thr	ttc tct tat Phe Ser Tyr 70	ggc gtg ca Gly Val Gl	g tgc ttt tcc n Cys Phe Ser 75	aga tac cca Arg Tyr Pro 80	240
65 gac cat atg aac Asp His Met Ly:		c ttt ttc aa o Phe Phe Ly	ag agc gcc atg ys Ser Ala Met 90	ccc gag ggc Pro Glu Gly 95	288
tat gtg cag ga Tyr Val Gln Gl 10	g aga acc ato u Arg Thr Ilo	c ttt ttc ac e Phe Phe L 105	aa gat gac ggg ys Asp Asp Gly	aac tac aag Asn Tyr Lys 110	336
acc cgc gct ga Thr Arg Ala Gl 115		c gaa ggt g e Glu Gly A 120	ac acc ctg gto sp Thr Leu Val 125	g aat aga atc L Asn Arg Ile S	384
gag ctg aag gg Glu Leu Lys Gl 130	c att gac tt y Ile Asp Ph 13	1-	at gga aac at sp Gly Asn Ile 140	t ctc ggc cac e Leu Gly His	432
aag ctg gaa ta Lys Leu Glu Ty	ac aac tat aa yr Asn Tyr As 150	ac tcc cac a sn Ser His <i>P</i>	aat gtg tac at Asn Val Tyr Il 155	c atg gcc gac e Met Ala Asp 160	480
145 aag caa aag aa Lys Gln Lys A		y3 var 110	ttc aag atc ag Phe Lys Ile Ar 170	a cac aac att g His Asn Ile 175	528
Glu Asp Gly S		tg gcc gac eu Ala Asp 185	cat tat caa ca His Tyr Gln Gl	ng aac act cca In Asn Thr Pro 190	576
		tc ctc cca eu Leu Pro 200	gac aac cat ta Asp Asn His Ty 20	ac ctg tcc acc yr Leu Ser Thr 05	624
cag tct gcc c Gln Ser Ala I	eu Ser Lys F	gat ccc aac Asp Pro Asn 215	gaa aag aga g Glu Lys Arg A 220	ac cac atg gtc sp His Met Val	672
Leu Leu Glu			atc aca cat g Ile Thr His G 235	gc atg gac gag ly Met Asp Glu 240	720
225					732
ctg tac aag Leu Tyr Lys	tga				

<211> 243 <212> PRT <213> Artificial Sequence

Met Thr Thr Thr Thr Ser Lys Gly Glu Glu Leu Phe Thr Gly Val

Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe

Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr

Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr

Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro

Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly

Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys

Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile

Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His 135

Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp

Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile

Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro

Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr

Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val 215

Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 225

Leu Tyr Lys

<210> 107 <211> 732

<212> DNA

<213> Artificial Sequence

<223> Description of Artificial Sequence: Trp(TGG)5GFP

<220>

<221> CDS

<222> (1)..(732)

WO 00/42215 - CX - PCT/AU00/00008

<400 atg Met '			tgg Trp	tgg Trp 5	tgg Trp	agc Ser	aag Lys	ggc Gly	gag Glu 10	gaa Glu	ctg Leu	ttc Phe	act Thr	ggc Gly 15	gtg Val	48
gtc (	cca Pro	att Ile	ctc Leu 20	gtg Val	gaa Glu	ctg Leu	gat Asp	ggc Gly 25	gat Asp	gtg Val	aat Asn	Gly	cac His 30	aaa Lys	ttt Phe	96
tct Ser	gtc Val	agc Ser 35	gga Gly	gag Glu	ggt Gly	gaa Glu	ggt Gly 40	gat Asp	gcc Ala	aca Thr	tac Tyr	gga Gly 45	aag Lys	ctc Leu	acc Thr	144
ctg Leu	aaa Lys 50	ttc Phe	atc Ile	tgc Cys	acc Thr	act Thr 55	gga Gly	aag Lys	ctc Leu	cct Pro	gtg Val 60	cca Pro	tgg Trp	cca Pro	aca Thr	192
ctg Leu 65	gtc Val	act Thr	acc Thr	ttc Phe	tct Ser 70	tat Tyr	ggc Gly	gtg Val	cag Gln	tgc Cys 75	ttt Phe	tcc Ser	aga Arg	tac Tyr	cca Pro 80	240
gac Asp	cat His	atg Met	aag Lys	cag Gln 85	cat His	gac Asp	ttt Phe	ttc Phe	aag Lys 90	agc Ser	gcc Ala	atg Met	ccc Pro	gag Glu 95	ggc Gly	288
tat Tyr	gtg Val	cag Gln	gag Glu 100	aga Arg	acc Thr	atc Ile	ttt Phe	ttc Phe 105	aaa Lys	gat Asp	gac Asp	Gly	aac Asn 110	tac Tyr	aag Lys	336
acc Thr	cgc Arg	gct Ala 115	gaa Glu	gtc Val	aag Lys	ttc Phe	gaa Glu 120	ggt Gly	gac Asp	acc Thr	ctg Leu	gtg Val 125	A311	aga Arg	atc Ile	384
gag Glu	ctg Leu 130	Lys	ggc Gly	att Ile	gac Asp	ttt Phe 135	aag Lys	gag Glu	gat Asp	gga Gly	aac Asn 140	116	ctc Leu	ggc	cac His	432
Lys 145	Leu	Glu	Tyr	Asn	Tyr 150	Asn	Ser	HIS	Asn	155	ı	116		gcc Ala	160	480
aag Lys	caa Gln	aag Lys	aat Asn	ggc Gly 165	Ile	aag Lys	gtc Val	aac Asn	Phe 170	r PA a	ato : Ile	aga Arg	cac His	aac Asn 175	*	528
gag Glu	gat Asp	gga Gly	tcc Ser 180	Val	cag Gln	ctg Leu	gcc Ala	gac Asp 185	HIS	tat Tyr	caa Glr	ı caç ı Glr	g aac n Asr 190	1 1111	cca Pro	576
atc Ile	ggc Gly	gac Asp 195	Gly	cct Pro	gtg Val	cto Lev	cto Lev 200	ı Pro	gac Asp	aac Asr	cat His	tao Tyi 205	r ne	g tcc ı Ser	acc Thr	624
cag Gln	tct Ser 210	: Ala	ctç Lev	tct Ser	aaa Lys	gat Asp 215	Pro	c aad o Asr	gaa n Glu	a aaq ı Lys	g aga s Arc 220	i vəf	c cac o Hi:	atç s Met	g gtc Val	672
ctg Leu 225	Let	g gag ı Glu	ttt Phe	gto Val	g acc L Thi 230	c Ala	gct a Ala	t ggg a Gly	g ato y Ile	e Thi	r HI:	t gge s Gl	c ate y Me	g gad t Asp	gag Glu 240	720
		c aaq c Lys		a												732

PCT/AU00/00008

<210> 108 <211> 243 <212> PRT <213> Artificial Sequence Met Trp Trp Trp Trp Ser Lys Gly Glu Glu Leu Phe Thr Gly Val

Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe

Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr

Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr

Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 65 70 75 80

Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly

Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 105

Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile

Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His

Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp

Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile

Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro 185

Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr

Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val

Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 230 225

Leu Tyr Lys

<210> 109

<211> 732 <212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Tyr(TAT)5GFP

<220> <221> CD <222> (1	s )(73	2) ·												
<400> 10 atg tat Met Tyr 1	tat ta Tyr Ty	r Tyr 5	Tyr 5	er ny	3 01	. y	10					15		48
gtc cca Val Pro	Ile Le	c gtg u Val 0	gaa c Glu L	tg ga eu As	D G	jc g Ly A 25	at o	gtg Val	aat Asn	ggg Gly	cac His 30	aaa Lys	ttt Phe	96
tct gtc Ser Val	agc gg Ser Gl 35	a gag y Glu	ggt g Gly G	itu Gi	gt ga Ly As 10	at g sp A	cc a	aca Thr	tac Tyr	gga Gly 45	aag Lys	ctc Leu	acc Thr	144
ctg aaa Leu Lys 50	ttc at Phe Il	c tgc e Cys	acc a	ct gg Thr Gl 55	ga aa ly L	ag c ys I	etc Leu	cct Pro	gtg Val 60	cca Pro	tgg Trp	cca Pro	aca Thr	192
ctg gtc Leu Val 65	act ac	cc ttc nr Phe	tct i Ser i	tat go Tyr G	gc g ly V	tg d al (	cag Gln	tgc Cys 75	ttt Phe	tcc Ser	aga Arg	tac Tyr	cca Pro 80	240
gac cat Asp His	atg aa Met L	ag cag ys Gln 85	HIS	gac t Asp P	tt t he P	tc a	aag Lys 90	agc Ser	gcc Ala	atg Met	ccc Pro	gag Glu 95	ggc Gly	288
tat gtg Tyr Val	Gln G	ag aga lu Arg 00	acc Thr	atc t Ile P	He r	tc he .05	aaa Lys	gat Asp	gac Asp	Gly	aac Asn 110	tac Tyr	aag Lys	336
acc cgc Thr Arg	gct g Ala G 115	aa gto lu Val	aag Lys	Phe G	aa g lu G .20	ggt Sly	gac Asp	acc Thr	ctg Leu	gtg Val 125	aat Asn	aga Arç	atc   Ile	384
gag cto Glu Leo 130	Lys G	gc att	t gac e Asp	ttt a Phe I 135	ag g ys (	gag Glu	gat Asp	gga Gly	aac Asn 140		cto Lev	ggo Gly	c cac / His	432
aag cto Lys Le 145	g gaa t ı Glu I	ac aa Yyr As:	c tat n Tyr 150	aac t Asn S	cc ( Ser !	cac	aat Asn	gtg Val 155		ato Ile	ato e Met	g gco : Ala	gac a Asp 160	480
aag ca Lys Gl	a aag a n Lys <i>l</i>	aat gg Asn Gl 16	у тте	aag ( Lys '	gtc ( Val :	aac Asn	ttc Phe 170		g ato	e Ar	a cad g His	c aa s As: 17	c att n Ile 5	528
gag ga Glu As	p Gly	tcc gt Ser Va 180	g cag l Gln	ctg Leu	gcc Ala	gac Asp 185	cat	tai Ty	t caa r Gl	a ca n Gl	g aa n As: 19	c ac n Th O	t cca r Pro	576
atc gg Ile Gl	c gac y Asp 195	ggc co Gly Pr	t gtg o Val	ctc Leu	ctc Leu 200	cca Pro	gac Asp	c aa o As:	c ca n Hi	t ta s Ty 20	c ct r Le 5	g to u Se	c acc r Thr	624
cag to Gln Se 21	t gcc er Ala	ctg to Leu Se	ct aaa er Lys	gat Asp 215	ccc Pro	aac Asn	gaa Glu	a aa u Ly	g ag s Ar 22	9 -	c ca p Hi	c at s Me	g gtc et Val	672
ctg ct Leu Le 225	g gag eu Glu	ttt g	tg acc al Thr 230	ALA	gct Ala	ggg Gly	ate Ile	c ac e Th 23		it gg .s Gl	gc at Ly Me	g ga	ac gag sp Glu 240	720

ctg tac aag tga . Leu Tyr Lys

<210> 110

<211> 243

<212> PRT

<213> Artificial Sequence

Met Tyr Tyr Tyr Tyr Tyr Ser Lys Gly Glu Glu Leu Phe Thr Gly Val

Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe

Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr

Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr

Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 65 70 80

Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly

Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 100

Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile

Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His

Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp

Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 165

Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro 185

Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr 200

Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val

Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 230

Leu Tyr Lys

<210> 111

<211> 732

<212> DNA

<213> Artificial Sequence

<220> <223> Description of Artificial Sequence: Tyr(TAC)5GFP <220> <221> CDS <222> (1)...(732) atg tac tac tac tac agc aag ggc gag gaa ctg ttc act ggc gtg <400> 111 48 Met Tyr Tyr Tyr Tyr Ser Lys Gly Glu Glu Leu Phe Thr Gly Val gtc cca att ctc gtg gaa ctg gat ggc gat gtg aat ggg cac aaa ttt Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe 96 tet gtc agc gga gag ggt gaa ggt gat gcc aca tac gga aag ctc acc 144 Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr ctg aaa ttc atc tgc acc act gga aag ctc cct gtg cca tgg cca aca Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr 192 ctg gtc act acc ttc tct tat ggc gtg cag tgc ttt tcc aga tac cca 240 Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro gac cat atg aag cag cat gac ttt ttc aag agc gcc atg ccc gag ggc 288 Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly 85 tat gtg cag gag aga acc atc ttt ttc aaa gat gac ggg aac tac aag 336 Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 105 acc cgc gct gaa gtc aag ttc gaa ggt gac acc ctg gtg aat aga atc 384 Thr Arg Ála Glu Val Lys Phe Glu Gly Ásp Thr Leu Val Asn Arg Ile 125 120 115 gag ctg aag ggc att gac ttt aag gag gat gga aac att ctc ggc cac Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His 432 135 130 aag ctg gaa tac aac tat aac tcc cac aat gtg tac atc atg gcc gac 480 Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 145 aag caa aag aat ggc atc aag gtc aac ttc aag atc aga cac aac att 528 Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile gag gat gga tcc gtg cag ctg gcc gac cat tat caa cag aac act cca 576 Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro 185 atc ggc gac ggc cct gtg ctc ctc cca gac aac cat tac ctg tcc acc 624 Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr 200 cag tot goo otg tot aaa gat ooc aac gaa aag aga gac cac atg gto 672 Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val

720

732

ctg ctg gag ttt gtg acc gct gct ggg atc aca cat ggc atg gac gag Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 235 ctg tac aag tga Leu Tyr Lys <210> 112 <211> 243 <212> PRT <213> Artificial Sequence Met Tyr Tyr Tyr Tyr Ser Lys Gly Glu Glu Leu Phe Thr Gly Val <400> 112 Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 65 Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 105 Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro 185 Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 235 230 Leu Tyr Lys

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ctg aaa ttc atc tgc acc act gga aag ctc cct gtg cca tgg cca aca Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr 50 55	192
ctg gtc act acc ttc tct tat ggc gtg cag tgc ttt tcc aga tac cca Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 80 65	240
gac cat atg aag cag cat gac ttt ttc aag agc gcc atg ccc gag ggc Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly 95	288
tat gtg cag gag aga acc atc ttt ttc aaa gat gac ggg aac tac aag Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys 100 105	336
acc cgc gct gaa gtc aag ttc gaa ggt gac acc ctg gtg aat aga atc Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile 115 120	384
gag ctg aag ggc att gac ttt aag gag gat gga aac att ctc ggc cac Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His 130	432
aag ctg gaa tac aac tat aac tcc cac aat gtg tac atc atg gcc gac Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp 145 150 150	480
aag caa aag aat ggc atc aag gtc aac ttc aag atc aga cac aac att Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile 175	528
gag gat gga toc gtg cag otg goo gao cat tat caa cag aac act coa Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro 180 185	576
atc ggc gac ggc cct gtg ctc ctc cca gac aac cat tac ctg tcc acc Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr	624

205 200 195 cag tot goo ctg tot aaa gat coo aac gaa aag aga gac cac atg gto 672 Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val 215 210 ctg ctg gag ttt gtg acc gct gct ggg atc aca cat ggc atg gac gag 720 Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 732 ctg tac aag tga Leu Tyr Lys <210> 116 <211> 243 <212> PRT <213> Artificial Sequence Met Val Val Val Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro 65 70 80 Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val 215 Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu 230

Leu Tyr Lys

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gtc Val	cca Pro	att Ile	ctc Leu 20	gtg Val	gaa Glu	ctg Leu	gat Asp	ggc Gly 25	gat Asp	gtg Val	aat Asn	Gly ggg	cac His 30	aaa Lys	ttt Phe	96 -
tct Ser	gtc Val	agc Ser 35	gga Gly	gag Glu	ggt Gly	gaa Glu	ggt Gly 40	gat Asp	gcc Ala	aca Thr	tac Tyr	gga Gly 45	aag Lys	ctc Leu	acc Thr	144
ctg Leu	aaa Lys 50	ttc Phe	atc Ile	tgc Cys	acc Thr	act Thr 55	gga Gly	aag Lys	ctc Leu	cct <sup>.</sup> Pro	gtg Val 60	cca Pro	tgg Trp	cca Pro	aca Thr	192
ctg Leu 65	gtc Val	act Thr	acc Thr	ttc Phe	tct Ser 70	tat Tyr	ggc Gly	gtg Val	cag Gln	tgc Cys 75	ttt Phe	tcc Ser	aga <sup>.</sup> Arg	tac Tyr	cca Pro 80	240
	cat His	atg Met	aag Lys	cag Gln 85	cat His	gac Asp	ttt Phe	ttc Phe	aag Lys 90	agc Ser	gcc Ala	atg Met	ccc Pro	gag Glu 95	ggc Gly	288
tat Tyr	gtg Val	cag Gln	gag Glu 100	aga Arg	acc Thr	atc Ile	ttt Phe	ttc Phe 105	aaa Lys	gat Asp	gac Asp	ggg Gly	aac Asn 110	tac Tyr	aag Lys	336
acc Thr	cgc Arg	gct Ala 115	Glu	gtc Val	aag Lys	ttc Phe	gaa Glu 120	ggt Gly	gac Asp	acc Thr	ctg Leu	gtg Val 125	aat Asn	aga Arg	atc Ile	384
gag Glu	ctg Leu 130	Lys	ggc	att	gac Asp	ttt Phe 135	ьуѕ	gag Glu	gat Asp	gga Gly	aac Asn 140		ctc Leu	ggc	cac His	432
aag Lys 145	Leu	gaa Glu	tac Tyr	aac Asn	tat Tyr 150	ASI	tcc Ser	cac His	aat Asn	gtg Val 155	- 1 -	atc Ile	atg Met	gcc Ala	gac Asp 160	480
aaç Lys	caa Glr	aaq Lys	g aat s Asr	ggc Gly 165	, ITe	aaç Lys	gtc Val	aac Asr	ttc Phe 170	: Lys	ato Ile	aga Arg	Cac	aac Asr 175	att lle	528
gaç Glı	g gat ı Asp	gga Gly	a tco y Sei 180	r Val	g caç L Glr	g cto Lev	g gcc ı Ala	gac Asp 185	) III.	tat Tyr	caa Glr	a caç n Glr	aad Asi 190	act n Thi	cca r Pro	576

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cag :	tct Ser 210	gcc Ala	ctg Leu	tct Ser	Lys	gat Asp 215 <sub>.</sub>	ccc Pro	aac Asn	gaa G1u	aag Lys	aga Arg 220	gac Asp	cac His	atg Met	gtc Val	672
ctg Leu 225	ctg Leu	gag Glu	ttt Phe	gtg Val	acc Thr 230	gct Ala	gct Ala	ggg Gly	atc Ile	aca Thr 235	cat His	ggc Gly	atg Met	gac Asp	gag Glu 240	720
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_			100					105	•			Gly	110			
Thr	Arç	Ala 115	Glu	Val	Lys	Phe	Glu 120	Gly	/ Asp	Thr	Lev	val 125	Asn	Arg	Ile	
Glu	Let 130		s Gly	, Ile	Asp	Phe 135	Lys	Glu	a Asp	Gly	Asr 140	ı Ile	Leu	Gly	His	
Lys 145		ı Glı	1 Туг	Asr	Туг 150	Asr	Ser	His	s Asr	1 Val	1 Туз 5	: Ile	Met	: Ala	160	
Lys	Gļr	Ly:	s Asr	Gly 165	ı Ile	Lys	val	Ası	n Phe 170	э <b>Ly</b> : Э	s Ile	e Arg	His	175	ı Ile	
Glu	ı Ası	o Gl	y Sei 180	r Val	L Glr	ı Lev	ı Ala	a Ası	o His	з Ту	r Gl	n Glr	190	n Thr	Pro	
Ile	e Gl	y As <sub>i</sub> 19		y Pro	o Vai	l Lev	Lei 200	ı Pro	o Ası	p As	n Hi	s Ty:	Lev	ı Sei	r Thr	
Glr	n Se 21		a Le	u Se:	r Ly:	s As <sub>l</sub>	o Pro	o As	n Gl	u Ly	s Ar 22	g Ası O	o Hi	s Met	t Val	

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Gln	tct Ser 210		ctg Leu	tct Ser	aaa Lys	gat Asp 215	ccc Pro	aac Asn	gaa Glu	aag Lys	aga Arg 220	gac Asp	cac His	atg Met	gtc Val	672
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Lys 145		u Gl	u Ty	r As	n Ty 15	r Ası O	n Ser	c Hi:	s Asr	va. 15	1 Ту: 5	r Il	e Me	t Ala	160	
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Gl	u As	p Gl	y Se. 18	r Va	ı Gl	n Le	u Ala	a As 18	p Hi: 5	з Ту	r Gl	n Gl	n As 19	n Th:	r Pro	
Il	e Gl	y As 19	sp G1 95	y Pr	o Va	ıl Le	u Lei 20	u Pr O	o As	p As	n Hi	s Ty 20	r Le 5	u Se	r Thr	

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- CXXV -

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- cxxvi -

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Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe 50 60

Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln 65 70 75 80

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg 85 90 95

Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile 115 120 125

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn 130 135

Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly 145 150 150

Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val 165 170 175

Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro 180 180 190

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser 195 200 205

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## INTERNATIONAL SEARCH REPORT

International application No. PCT/AU 00/00008

A.	CLASSIFICATION OF SUBJECT MATTER							
Int Cl <sup>7</sup> :	C12Q 1/68							
According to	International Patent Classification (IPC) or to bot	h national classification and IPC						
В.	FIELDS SEARCHED							
Minimum docu AS ABOVE	Minimum documentation searched (classification system followed by classification symbols) AS ABOVE							
Documentation AS BELOW	searched other than minimum documentation to the ex	ctent that such documents are included in t	the fields searched					
CA, Medline	base consulted during the international search (name of Biosis, WPID preference/use/usage/efficiency); tandem repe		terms used)					
C.	DOCUMENTS CONSIDERED TO BE RELEVAN	Т						
Category*	Citation of document, with indication. where ap	propriate, of the relevant passages	Relevant to claim No.					
A	Kurland, GC. N Codon Bias and Gene Expr 285(2):165-169	All						
, <b>A</b>	Sharp PM et al. Codon usage: mutationa or both? Biochem Soc Trans 1993. 21(4).	All						
A	A AU 56657/96. BIOGEN MV. 9/10/86							
	Further documents are listed in the continuation of Box C	X See patent family an	nex					
* Special categories of cited documents:  "A" document defining the general state of the art which is not considered to be of particular relevance  "E" earlier application or patent but published on or after the international filing date  "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) document referring to an oral disclosure, use, exhibition or other means  "P" document defining the general state of the art which is not considered to be of particular relevance; the claimed invention canno be considered novel or cannot be considered to involve an inventive step when the document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document combined with one or more other such documents, such combination being obvious to a person skilled in the art document member of the same patent family								
	al completion of the international search	Date of mailing of the international search	ch report					
7 February 2		1 8 FEB 2000						
V	ing address of the ISA/AU  PATENT OFFICE	Authorized officer	•					
PO BOX 200, V E-mail address:	AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929  GILLIAN ALLEN Telephone No.: (02) 6283 2266							

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No. PCT/AU 00/00008

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report  AU 56657/96	Patent Family Member	
	WO 86/05810	
	EP 215110	·
	US 5242811	
	·	
		END OF ANNEX

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